Universidad de Huelva

Departamento de Química "Profesor José Carlos Vílchez Martín"

Valorization of residual strawberry extrudate througt obtaining high added value compounds and methane production by anaerobic digestion

Memoria para optar al grado de doctor
presentada por:

Juan Cubero Cardoso

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Valorización del extrusionado de fresa residual mediante la obtención de compuestos de alto valor añadido y producción de metano por digestión anaerobia

Tesis Doctoral presentada por
Juan Cubero Cardoso
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Table of Contents

Resumen

De acuerdo con la base de datos estadísticos de la FAO (Food and Agriculture Organization), la producción mundial de fresa en 2017 fue de 9 millones de toneladas, siendo España uno de los países con mayor producción, un 3,9 % del total, lo que representa una producción de más 360.000 toneladas/año. De hecho, del total de la cantidad producida a nivel europeo, el 21 % corresponde a la producción que ha aportado España. Según el Ministerio de Agricultura, Pesca y Alimentación de España, Andalucía es la comunidad autónoma con el mayor índice de producción de fresa, siendo su porcentaje de producción de un 97 % del total nacional. Especificando aún más, se observa que la provincia de **Huelva**, es la que presenta la mayor producción de fresa, con un 99,7 % de la producción dentro de Andalucía. El sector de la fresa constituye un motor importante en el desarrollo de la economía onubense y andaluza, ya que, en total, cerca de 2.000 explotaciones se dedican a este cultivo en Andalucía, generando más de 60.000 jornales al año.

Durante los últimos años, se ha ido desarrollando un creciente interés por el consumo de alimentos saludables con un alto contenido en compuestos fenólicos, debido a sus propiedades antioxidantes, antiinflamatorias, antimicrobianas, etc. La fresa (F*ragaria x ananassa*) es un fruto con un alto contenido en compuestos fenólicos, con importantes beneficios para la salud humana. Además de compuestos fenólicos, este fruto presenta una elevada concentración de fibra dietética, tales como hemicelulosa, celulosa y pectinas, y pequeñas cantidades de proteínas y ácidos grasos, así como un importante contenido de vitamina C y ácido fólico, compuestos todos ellos de indudables beneficios para la salud. Estudios recientes han asociado el consumo de fresas y sus componentes antioxidantes con mejoras sobre la salud, como la prevención de la inflamación vascular, el estrés oxidativo, enfermedades cardiovasculares, diabetes, cáncer u obesidad.

La fresa se utiliza no solo para su consumo directo por sus excelentes propiedades nutricionales, sino que también se emplea para la fabricación de otros productos derivados de la transformación del fruto, tales como mermeladas, yogures y otros derivados lácteos, bebidas refrescantes sin alcohol, helados, pasteles, sustancias aromatizantes, etc. Concretamente, alrededor del 21 % de la producción total de fresa es destinada a la obtención de estos productos. Durante el procesado industrial para la obtención de este concentrado, las fresas son prensadas y extrusionadas mediante un tornillo sin fin y tamices con diferentes luces de mallas. En este proceso, la pulpa y la parte líquida, que constituyen el concentrado de fresa, se separan de la parte fibrosa y los aquenios generando una fracción residual, que se denomina **extrusionado de fresa**.

La fracción residual extrusionada de la fresa puede alcanzar alrededor del 7 % en peso de todas las fresas manufacturadas, las cuales deben ser procesadas y tratadas adecuadamente debido a su alta carga orgánica, y en definitiva a su alto poder contaminante.

Actualmente, tal y como queda recogido en la Directiva Marco de Residuos 2018/851 modifica de la Directiva 2008/98/CE, los Estados miembros de la Unión Europea deben apoyar el uso de tratamientos que permitan la valorización de los residuos, priorizando las actuaciones en consonancia con la jerarquía de prevención, reutilización, valorización y eliminación. Asimismo, y con el objetivo de alcanzar una sociedad en la que prevalezca la valorización, no se debe fomentar el vertido o la incineración de dichos residuos, siempre que sea posible. Por otra parte, establece la necesidad de reducir progresivamente y eliminar a medio plazo el vertido de materia orgánica en los vertederos, mediante el empleo de tratamientos que permitan su valorización, siendo simultáneamente respetuosos con el medio ambiente y compatibles con estrategias de separación en origen y recuperación de la máxima cantidad de productos de alto valor añadido que sea posible. En concreto, la Unión Europea ha marcado como objetivo reducir la cantidad de residuos alimentarios depositados en vertederos en un 50 % para 2030, respecto a la depositada en el año 2015.

Para aprovechar los principales componentes del extrusionado de fresa (celulosa, hemicelulosa y lignina) y recuperar compuestos de alto valor añadido (compuestos bioactivos) se requiere un tratamiento previo que permita romper las barreras físicas y químicas de su complejo entramado estructural. Con este tratamiento previo se debería aumentar la accesibilidad a dichos compuestos, provocando la solubilización de los compuestos de interés, como son los compuestos fenólicos y los azúcares, posibilitando así la separación de las fases (sólida y líquida), obteniéndose una fácil recuperación de compuestos de alto valor añadido. Existen diferentes métodos de extracción entre los cuales se encuentra el pretratamiento hidrotérmico, ya sea a temperaturas y presiones bajas y/o medias, o con vapor a alta presión y temperatura y con descompresión rápida, llamado “steam-explosion”.

En el caso del residuo resultante del prensado y extrusionado de la fresa, en esta Tesis Doctoral se ha realizado un estudio con diferentes pretratamientos hidrotérmicos, en distintas condiciones de presión y temperatura. Tras el posterior pretratamiento hidrotérmico, se genera una fase líquida, rica en carbohidratos fácilmente degradables, y compuestos fenólicos de alto valor añadido que, por su carácter antioxidante, podrían ser de interés para la industria farmacéutica y alimentaria. Tras la realización de estos pretratamientos hidrotérmicos y una posterior separación de las fases resultantes, se propone un proceso de purificación de compuestos fenólicos haciendo uso de una resina adsorbente ya utilizada por la industria alimentaria llamada Amberlite XAD 16.

Los compuestos de alto valor añadido obtenidos tras los pretratamientos hidrotérmicos en distintas condiciones de presión y temperatura, podrían utilizarse como comonómeros para un proceso llamado vulcanización inversa. Recientemente, se han reportado en bibliografía varios estudios que demuestran la posibilidad de la utilización de algún residuo agroalimentario para su uso como comonómero en el proceso de vulcanización inversa. Usándose para la fabricación de dichos polímeros azufre elemental (S_8) proveniente de los procesos de refino de petróleo. El uso de copolímeros de origen natural provenientes de residuos de la industria agroalimentaria conduciría a la obtención de un material polimérico que ofrecería un alto grado de biodegradabilidad, poder antioxidante y propiedades antimicrobianas.

Para cerrar el ciclo de valorización del extrusionado de fresa, la biomasa resultante tras el pretratamiento hidrotérmico y la purificación de compuestos fenólicos, debe ser tratada adecuadamente ya que aún seguiría conteniendo una gran cantidad de materia orgánica. La fase sólida generada tras el pretratamiento hidrotérmico y la fase líquida desfenolizada obtenida tras la purificación son fuentes ricas en carbohidratos solubles, por lo que podrían ser un substrato muy adecuado para la generación de biogás mediante el proceso de digestión anaerobia controlada. Los procesos biológicos anaerobios son unos de los procesos más efectivos para el tratamiento y aprovechamiento de residuos de carácter orgánico. En éstos la materia orgánica del sustrato se transforma en biogás, mezcla de dióxido de carbono (CO_2, 30-40 %) y metano (CH_4, 60-70 %), cuyo elevado poder calorífico (5000-6000 kcal/m^3) permite su utilización como biocombustible o su uso para la generación de energía eléctrica y térmica mediante cogeneración. Todas estas razones han impulsado el estudio de la influencia de pretratamientos de hidrólisis hidrotérmica para así tratar de superar las dificultades que se podrían encontrar al procesar anaeróbicamente el residuo procedente del extrusionado de fresa sin tratamientos previos con un alto contenido en compuestos fenólicos.

En primer lugar, se han estudiado diferentes pretratamientos hidrotérmicos al extrusionado de fresa en un rango entre 90 °C y 200 °C con y sin adición de ácido acético para favorecer la solubilización de la materia orgánica. Se concluye que el pretratamiento hidrotérmico óptimo para la obtención de compuestos de alto valor agregado, como azúcares y compuestos fenólicos, es el llevado a cabo a una temperatura de 150 °C durante 60 minutos y sin adición de ácido acético.

Tras estos resultados, se propusieron estudios de valorización con el mismo extrusionado de fresa. Se aplicaron tres pretratamientos hidrotérmicos, la adsorción de compuestos fenólicos con resina Amberlite XAD 16 y un estudio de biodegradabilidad

mediante el proceso de digestión anaeróbica por lotes en régimen discontinuo mediante ensayos de potencial bioquímico de metano (Biochemical Methane Potential, BMP) de la biomasa remanente. El primer estudio se realizó a 150 ° C durante 60 minutos porque era el pretratamiento más óptimo obtenido con una primera muestra de extrusionado de fresa. El segundo y tercer estudio se llevaron a cabo a 170 °C durante 60 minutos y el tratamiento de steam explosion se incrementó a 220 °C durante 5 minutos, respecto el estudio anterior que solo llegó a 200 °C. Se concluyó que los estudios con pretratamientos hidrotermales a 170 °C y 220 °C fueron los más óptimos en la valorización del extrusionado de fresa mediante la obtención de compuestos fenólicos y en la producción de metano. Posteriormente, se eligieron estos dos pretratamientos hidrotérmicos a 170 °C y 220 °C para ampliar el estudio de la digestión anaeróbica, en este caso, en régimen semicontinuo utilizando diferentes velocidades de carga orgánica (OLR). La digestión anaerobia semicontinua del extrusionado de fresa desfenolizado previamente tratado mediante steam explosion a 220 °C durante 5 minutos se mantuvo estable a una OLR de 0,5 g de sólidos volátiles (SV)/(L · d), con una tasa de producción de metano específica de 135 ± 11 mL de CH_4/(g SV · d). Por otro lado, para la digestión anaerobia semicontinua del extrusionado de fresa desfenolizado previamente tratado mediante pretratamiento hidrotermal a 170 °C durante 60 minutos, permitió obtener una tasa de producción de metano óptima y estable de 243 ± 34 mL CH_4/(g SV · d) a una OLR de 1,25 g SV/(L · d).

En estos estudios también se ha realizado un análisis económico que mostró que la combinación de pretratamiento hidrotérmico junto con la recuperación de compuestos fenólicos y la digestión anaerobia en condiciones estables, generó un precio de venta para el extracto fenólico de 0,610 €/g de equivalentes de ácido gálico para el pretratamiento hidrotérmico a 220 °C, y del 0,812 €/g de equivalentes de ácido gálico para el pretratamiento hidrotérmico a 170 °C. Se concluye que el pretratamiento por explosión de vapor a 220 °C fue más efectivo para la solubilización y recuperación de compuestos fenólicos, pero la mayor capacidad de tratamiento y producción de metano se logró para el tratamiento hidrotérmico a 170 °C. Por tanto, los resultados mostraron que el tratamiento a 170 °C puede considerarse como el más adecuado para la valorización integral del extrusionado de fresa.

Finalmente, se prepararon diferentes copolímeros mediante vulcanización inversa con azufre, aceite de ricino y un tercer compuesto químico, uno de los cuales fue el ácido cinámico, un compuesto bioactivo del extruido de fresa. Se ha demostrado que estos copolímeros tienen poder antioxidante debido a que el tercer compuesto químico tiene heteroátomos. Además, se ha observado que estos copolímeros tienen actividad antimicrobiana, especialmente en bacterias Gram + y levaduras como *Bacillus cereus* y *Saccharomyces cerevisiae*. Se concluye que este método de obtención de nuevos

copolímeros por vulcanización inversa sería una buena opción para el aprovechamiento de los compuestos bioactivos obtenidos del extrusionado de fresa mediante la aplicación de un pretratamiento hidrotérmico, y que sería una nueva línea de investigación que aún no ha sido explotada y en la que hay que profundizar en el futuro.

Palabras claves: Extrusionado de fresa, compuestos fenólicos, tratamiento hidrotérmico, digestión anaerobia, vulcanización inversa

Abstract

According to the statistical database of the FAO (Food and Agriculture Organization), the world strawberry production in 2017 was 9 million tons, Spain is one of the countries with the highest strawberry production, with 3.9 % of the total, whose production is more than 360,000 tons/year. In fact, of the total quantity produced at the European level, 21% corresponds to the production contributed by Spain. According to the Ministry of Agriculture, Fisheries and Food of Spain, Andalusia is the autonomous community with the highest strawberry production index, its production percentage being 97 % of the national total. Specifying even more, it is observed that the province of **Huelva** is the one with the highest strawberry production, representing 99.7 % of the production within Andalusia. The strawberry sector is a significant engine in the development of the Huelva and Andalusian economy as about 2,000 farms are dedicated to this crop in Andalusia, generating more than 60,000 job positions per year in total.

In recent years, there has been a growing interest in the consumption of healthy foods with a high content of phenol compounds due to their antioxidant, anti-inflammatory, antimicrobial properties, etc. Strawberry (*Fragaria x ananassa*) is a fruit with a high content of phenol compounds, with important benefits for human health. In addition, to phenol compounds, this fruit has a high concentration of dietary fiber, such as hemicellulose, cellulose, and pectins, and small amounts of proteins and fatty acids, as well as a higher content of vitamin C and folic acid, all of which are of undoubted benefits. to health. Recent studies have associated the consumption of strawberries and their antioxidant components with health improvements, such as the prevention of vascular inflammation, oxidative stress, cardiovascular disease, diabetes, cancer or obesity.

Strawberry is used not only for direct consumption due to its excellent nutritional properties, but is also used for the manufacture of other products derived from the transformation of the fruit, such as jams, yogurts, and other dairy derivatives, soft drinks without alcohol, ice cream, cakes, flavoring substances, etc. Specifically, around 21 % of the total strawberry production is used to obtain these products. During the industrial processing to obtain the strawberry concentrate for these by-products, the strawberries are pressed and extruded using twin-screws up to several sieves with different mesh sizes. In this process, the pulp and the liquid part, which constitute the strawberry concentrate, are separated from the fibers part and the achenes, generating a residual fraction, named **strawberry extrudate (SE)**. The strawberry extrudate fraction can reach around 7 % by weight of all manufactured strawberries, which must be managed and treated properly due to their high organic load and high polluting power.

Currently, as stated in the Waste Framework Directive 2018/851 amends Directive 2008/98 / EC, the Member States of the European Union must support the use of treatments that allow the recovery of waste, prioritizing actions in line with the hierarchy of prevention, reuse, recovery and disposal. Likewise, and in order to achieve a society in which recovery prevails, the dumping or incineration of the mentioned wastes should not be encouraged, whenever possible. On the other hand, it establishes the need to progressively reduce and eliminate in the medium term the dumping of organic matter in landfills. For this, it is proposed the use of treatments that allow valorization of these agro-wastes. However, these treatments should be simultaneously respectful of the environment and compatible with strategies of separation at source and recovery of the maximum amount of high added value compounds that is possible. Specifically, the European Union has set the objective of reducing the amount of food waste deposited on landfills by 50 % by 2030, compared to that deposited in 2015.

To take advantage of the main components of the strawberry extrudate (cellulose, hemicellulose, and lignin) and to recover high added value compounds (bioactive compounds), a previous treatment is required that allows breaking the physical and chemical barriers of its complex structural framework. With this previous treatment, the accessibility to the mentioned compounds should be increased, causing the solubilization of the compounds of interest, such as phenol compounds and sugars, thus allowing the separation of the phases (solid and liquid), and obtaining an easy recovery of high added value compounds. There are different extraction methods, such as hydrothermal pre-treatment, either at low and/or medium temperatures and pressures, as well as with steam at high pressure and temperature, and with rapid decompression, called “steam-explosion”.

In the case of the agro-wastes resulting from the pressed and extruded of the strawberry, in this Doctoral Thesis a study has been carried out with different hydrothermal pre-treatments, under different conditions of pressure and temperature. After the subsequent hydrothermal pre-treatment, it is generated a liquid phase, rich in easily degradable carbohydrates, and phenol compounds with high added value that, due to their antioxidant nature, could be of interest to the pharmaceutical and food industry. After carrying out these hydrothermal pre-treatments, it is proposed a purification process of phenol compounds using an adsorbent resin already used by the food industry called Amberlite XAD 16.

The high added value compounds obtained after hydrothermal pre-treatments, under different conditions of pressure and temperature, have been proposed for use as comonomers for a process called inverse vulcanization. Recently, several studies have been reported in the bibliography that demostrated the possibility of the use of some agro-waste, for its use as a comonomer in the inverse vulcanization process. Being used for the manufacture of the mentioned polymers elemental sulfur (S_8) from petroleum refining processes. The use of copolymers of natural origin that come residues of the agro-food industry would lead to obtaining a polymeric material that would offer a high degree of biodegradability, antioxidant power, and antimicrobial properties.

To close the recovery cycle of the strawberry extrudate, the remaining biomass after the hydrothermal pre-treatment and the purification of phenol compounds, must be treated adequately since it would still contain a large amount of organic matter. The solid phase generated after hydrothermal pre-treatment and the de-phenolized liquid phase obtained after purification are rich sources of soluble carbohydrates, which could be a very suitable substrate for the generation of biogas by controlled anaerobic digestion. Anaerobic biological processes are one of the most effective processes for the treatment and use of organic wastes. In these, the organic matter of the substrate is transformed into biogas, a mixture of carbon dioxide (CO_2, 30-40%) and methane (CH_4, 60-70%), whose high calorific value (5000-6000 kcal/m^3) allows its use as biofuel or its use for the generation of electrical and thermal energy through cogeneration. All these reasons have promoted the study of the influence of hydrothermal hydrolysis pre-treatments in order to try to overcome the difficulties that could be encountered when anaerobically processing the residue from strawberry extrudate without treatments with a high content of phenol compounds.

First, different hydrothermal pre-treatments of the strawberry extrudate have been studied in a range between 90 ° C and 200 ° C with and without the addition of acetic acid to favor the solubilization of organic matter. It is concluded that the optimal hydrothermal pre-treatment to obtain high added value compounds, such as sugars and phenol compounds, is carried out at a temperature of 150 ° C for 60 minutes and without the addition of acetic acid.

After these results, valorization studies were carried out with the same strawberry extrudate. Three hydrothermal pre-treatments were applied, the adsorption of phenol compounds with Amberlite XAD 16 resin and a study of biodegradability through the batch mode anaerobic digestion process in a discontinuous regime using biochemical methane potential tests (BMP) of the remaining biomass. The first study was carried out at 150 ° C for 60 minutes because it was the most optimal pre-treatment obtained with a first sample of strawberry extrudate. The second and third studies were carried out at 170 ° C for 60 minutes and the steam explosion treatment was increased to 220 ° C for 5 minutes, compared to the previous study that only reached 200 ° C. It was concluded that the studies with hydrothermal pre-treatments at 170 ° C and 220 ° C were the most optimal in the valorization of the strawberry extrudate by obtaining phenol compounds and in the production of methane. Subsequently, these two hydrothermal pre-treatments at 170 ° C and 220 ° C were chosen to expand the study of anaerobic digestion, in this case, in a semi-continuous regime using different organic loading rates (OLR). The semi-continuous anaerobic digestion of the de-phenolized strawberry extrudate previously treated by steam explosion at 220 ° C for 5 minutes was stable at an OLR of 0.5 g of volatile solids (VS) / (L · d), with a production rate of specific methane of 135 ± 11 mL of CH_4 / (g VS · d). On the other hand, the semi-continuous anaerobic digestion of the de-phenolized strawberry extrudate previously treated by hydrothermal pre-treatment at 170 ° C for 60 minutes, it allowed to obtain an optimal and stable methane production rate of 243 ± 34 mL CH_4 / (g VS · d) at an OLR of 1.25 g VS / (L · d).

In these studies, an economic analysis has also been carried out that showed that the combination of hydrothermal pre-treatment, phenol compounds recovery, and anaerobic digestion under stable conditions, generated a sale price for the phenol compounds extract of 0.610 EUR/g of equivalents of gallic acid for hydrothermal pre-treatment at 220 ° C, and 0.812 EUR/g gallic acid equivalents for hydrothermal pre-treatment at 170 ° C. It is concluded that the steam explosion pre-treatment at 220 ° C was more effective for the solubilization and recovery of phenol compounds, but the highest methane production and capacity of treatment were achieved for the hydrothermal treatment at 170 ° C. Therefore, the results showed that the treatment at 170 ° C can be considered as the most suitable for the integral valorization of the strawberry extrudate.

Finally, different copolymers were prepared by inverse vulcanization with sulfur, castor oil and a third chemical compound, one of which was cinnamic acid, a bioactive compound from strawberry extrudate. These copolymers have been shown to have antioxidant power because the third chemical compound has heteroatoms. Furthermore, these copolymers have been found to have antimicrobial activity, especially in Gram + bacteria and yeasts such as *Bacillus cereus* and *Saccharomyces cerevisiae*. It is concluded that this method of obtaining new copolymers by inverse vulcanization would be a good option for the use of bioactive compounds obtained from strawberry extrudate by applying a hydrothermal pre-treatment, and that it would be a new line of research that has not yet been exploited up to now and should be deepened in the future.

Keywords: Strawberry extrudate, phenol compounds, hydrothermal treatment, anaerobic digestion, inverse vulcanization

1

Valorization options of strawberry extrudate agro-waste

Abstract

This review summarizes and critically analyzes the different types of potential valorization options for strawberry extrudate in order to have a broader overview of the potential management of this waste. Animal feed is commonly used as a management option for the strawberry extrudate; however, most of the strawberry extrudate is disposed in landfills. Strawberry extrudate contains different bioactive compounds that encourage the use of an alternative management approach than landfilled. The present review offers a complete comparison, including the advantages and drawbacks of each reviewed technique, to facilitate the selection of the most suitable technology for the different valorization scenarios. This review has been structured in four sections: 1. Composition of the strawberry extrudate and strawberry specifically focused on their content in bioactive compounds. 2. The different techniques of extraction and purification of bioactive compounds. 3. The handling and management of the resulting biomass after the extraction process of bioactive compounds. 4. Inverse vulcanization an option for the bioactive compounds.

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1.1. Introduction

In 2016, 8 million tons of strawberries were produced in the world with a value of agricultural gross production of 17,739 million US$ (FAO 2020). Besides its market as fresh product, strawberry is also used to produce many types of by-products, due to its peculiar flavor and aroma. Strawberry by-products are mainly formulated from a strawberry concentrate. The most common technology to obtain the strawberry concentrate is by extrusion. Strawberries are extruded by twin-screws up to several sieves with different mesh sizes. The sieves retain a residual fraction formed by the fibers part and the achenes, named strawberry extrudate (**SE**), which accounts about 7 % of the manufactured strawberry (Gutiérrez et al. 2017).

Animal feed is commonly used as a management option for the strawberry extrudate, however, most of the strawberry extrudate is disposed in landfills, contributing to greenhouse emissions due to its high organic load (Serrano et al. 2013). Alternatives for strawberry extrudate management are required to avoid severe environmental impacts that cause landfills, such as negative effects on agricultural soil quality, polluting of aquatic ecosystems and atmospheric contamination (Aharonov-Nadborny et al. 2016).

Similar to strawberry, strawberry extrudate contains substances of high interest such as bioactive compounds. Some of these bioactive compounds have beneficial health effects on cardiovascular, neurological or cancerous disorders (Giampieri et al. 2012). Due to their health benefits, bioactive compounds have an economic interest for different commercial sectors, such as the pharmaceutical, food and chemical industries (Dias et al. 2017). Added to bioactive compounds, strawberry extrudate could be used to obtain other types of resources such as bioenergy (Dias et al. 2009; López de Dicastillo et al. 2016). It is also well known the high phenol composition in the achenes and in the pulp of the strawberry (Ariza et al. 2016).

A general biorefinery scheme as a management option for the strawberry extrudate should look for synergies between unitary processes of extraction of bioactive compounds, purification, and the management of the final biomass of the strawberry extrudate after extraction (**Figure 1.1**).

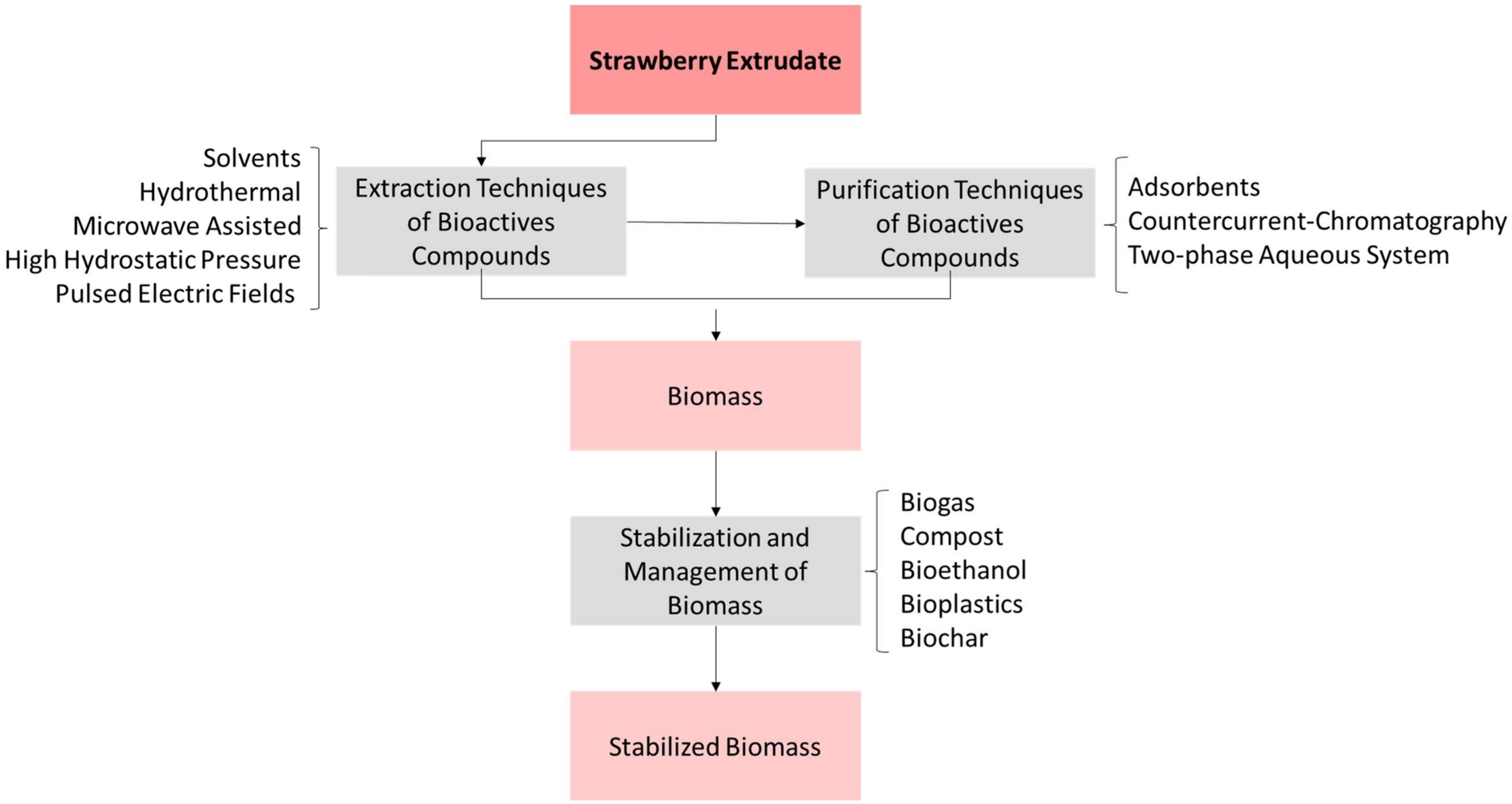

Figure 1.1. General scheme of a biorefinery approach as a valorization option for strawberry extrudate.

The extraction of bioactive compounds in agro-wastes materials, such as the strawberry extrudate can be performed through various extraction techniques (Wijngaard et al. 2012). The main objective at this step consists of solubilizing the compounds of interest, with less possible impurities and making it an economically profitable technique. Extraction techniques in literature can be clustered into two groups: conventional extraction techniques, e.g. hydrothermal treatments, which are widely used at lab and full scale (Fermoso et al. 2018), and in recent years, more innovative techniques, e.g. enzyme assisted extraction. Additionally, combined extraction techniques between conventional and innovative techniques are being carried out to achieve high extraction yield (Pérez-Loredo et al. 2017). All these extraction techniques will be revised and analyzed in the present chapter.

Any of these extraction techniques usually generate a liquid phase, with the bioactive compounds of interest, and a solid phase with a high amount of organic matter. After purification process of the liquid phase, a new liquid phase remains without the extracted bioactive compounds. Therefore, just the recovery of compounds of interest from the strawberry extrudate does not solve the problem of stabilization of the biomass of the strawberry extrudate and the use of a subsequent treatment is necessary for its stabilization (Rajendran et al. 2018). The liquid phase after purification and the solid phase must undergo a new treatment for stabilization. In addition, extraction and purification processes consume energy which should be valued. The main options for assessing and stabilizing biomass after the extraction and purification process of the bioactive compounds should be focused on obtaining bioenergy and other bioproducts of interest (Machineni 2019).

The present chapter aims to summarize the bioactive compounds present in strawberries, to summarizes and critically analyzes the different extraction and purification techniques for the recovery of these bioactive compounds, as well as the different options for the management and stabilization of the strawberry extrudate after the extraction process.

1.2. Bioactive compounds in strawberry extrudate and strawberries

1.2.1 Nutrients

Strawberry extrudate presents similar nutrients composition than strawberry (Rodríguez-Gutiérrez et al. 2019). The strawberry has high concentration of dietary fibers (2 g fibers/100 g raw strawberry), such as lignin, hemicellulose, cellulose, and pectin, containing small amounts of protein (0.4–0.5 g protein/100 g raw strawberry) and fat (0.1 g fat/100 g raw strawberry) (Sójka et al. 2013; Basu et al. 2014).

The strawberry contains high concentrations of vitamin C, contributing to 24 % to the antioxidant capacity of strawberries (Basu et al. 2014). The recommended daily intake of vitamins (100–150 mg/day) can be satisfied with an average of 100 g of strawberries per day (Kafkas et al. 2007). Furthermore, strawberry is a source of many other vitamins in smaller amounts, such as vitamin E, vitamin A, vitamin B6, vitamin K, thiamine, riboflavin, folate acid, and niacin (0.01–0.4 g vitamin/100 g raw strawberry) (Tulipani et al. 2008; Giampieri et al. 2012). Strawberry is also rich in manganese, potassium, and a good source of iodine, magnesium, copper, iron, and phosphorus (Giampieri et al. 2012; Basu et al. 2014).

The sugar composition of strawberries varies with the degree of maturity of the fruit (da Silva Pinto et al. 2008), being glucose, fructose, and sucrose the main sugars in strawberries (Giampieri et al. 2012). Sugars in strawberries are involved in the taste of the fruit and are responsible for the caloric value of the strawberries. Organic fatty acids such as citric acid, malic acid, succinic acid, tartaric acid, oxalic acid, and fumaric acid are ones of the response of the taste, texture, pH, and color of the strawberry, and can alter the sensory quality of this fruit (Dias et al. 2017).

1.2.2 Phytochemicals

Figure 1.2 shows a general scheme for the classification of phytochemical compounds that can be founded in the strawberry extrudate. Phytochemicals are widely studied, mainly due to the extensive types of compounds that have potential biological benefits in humans. The main phytochemicals in strawberries are the flavonoids, followed by the hydrolyzable tannins and the phenol acids and, as minor constituents, the condensed tannins (Giampieri et al. 2012).

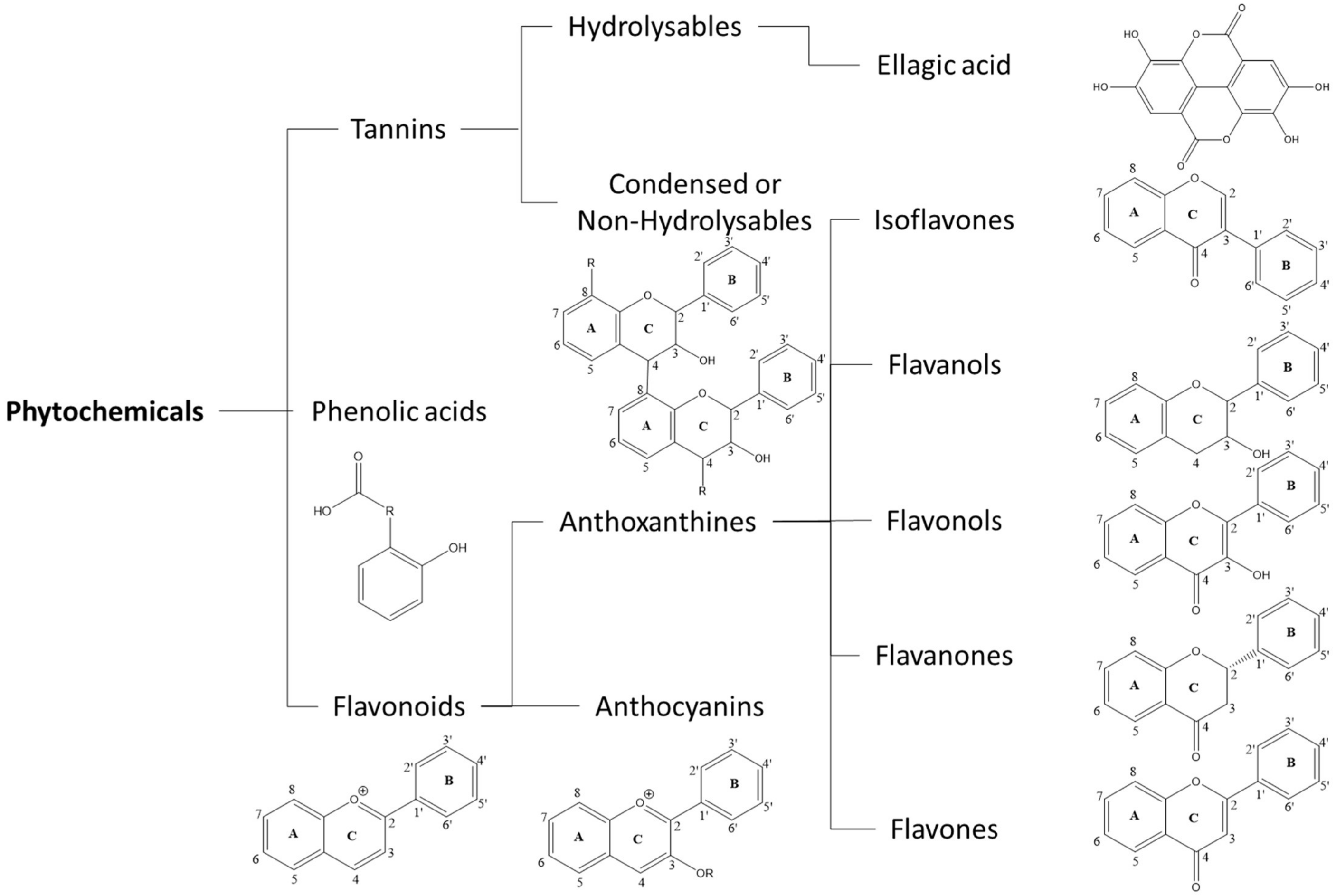

Figure 1.2. General scheme of phytochemicals contained in strawberries.

1.2.2.1 Flavonoids

Flavonoids are divided into two groups, i.e., anthocyanins, and anthoxanthines. Anthoxanthines, in turn, are grouped into five subclasses, i.e., flavones, flavonols, flavanones, flavanols, and isoflavones (Ochoa and Ayala 2004). The three main classes of flavonoids in strawberries are anthocyanins, flavonols, and flavanols (Robards et al. 1999).

The most relevant flavonoids present in strawberries are the anthocyanins due to their high concentration, approximately 20–47 mg/100 g raw strawberry (Basu et al. 2014). More than 25 different pigments of anthocyanins have been described in the different varieties of strawberries (Giampieri et al. 2012). Anthocyanins are responsible for the red color in strawberries (Basu et al. 2014). The most important anthocyanins of the strawberry belong to the family of pelargonidin aglycones and cyanidin aglycones (Böhm 1994; da Silva et al. 2007; da Silva Pinto et al. 2008). According to several studies, pelargonidin-3-glucoside is the dominant anthocyanin in strawberries (Aaby et al. 2005, 2007; Klopotek et al. 2005; da Silva et al. 2007; Basu et al. 2014). The interest in anthocyanins have recently increased because of its pharmacological and therapeutic properties (Giampieri et al. 2012). Anthocyanins have shown to positive effect toward reduction of coronary diseases, anticancer, antitumor, anti-inflammatory and anti-diabetic effects; as well as improving visual acuity and cognitive behavior (Garzón 2010). These therapeutic effects of anthocyanins are connected to their high antioxidant activity. In addition, anthocyanins can be used as a pigment in the food industry (Garzón 2010).

The second most important group of flavonoids in strawberries are flavonols, with approximately 1.5–3.4 mg/100 g raw strawberry (Hannum 2004; Giampieri et al. 2012; Shaik-Dashagirisaheb et al. 2013; Basu et al. 2014). The most important flavonols of the strawberry belong to the family of quercetin and kaempferol, being the quercetin-derivatives the most abundant flavonols in strawberries (Aaby et al. 2007; da Silva Pinto et al. 2008; Giampieri et al. 2012; Sójka et al. 2013). Quercetin, in particular, is a potent antioxidant, cytoprotective, and anti-inflammatory (Shaik-Dashagirisaheb et al. 2013).

Finally, the third group of flavonoids in strawberries are flavanols. Flavanols are the only class of flavonoids that do not naturally occur as glycosides. They are found in strawberries as monomeric compounds, such as catechins, and in polymeric form, which are called condensed or non-hydrolyzable tannins (da Silva Pinto et al. 2008; Giampieri et al. 2012). These compounds can be difficult to measure in the strawberry because they are usually presented as part of a complex mixture of phenol substances. Because of this, the amount of catechins present is sometimes overestimated (Hannum 2004). At low concentrations flavanols particularly the catechins, are used as sweetening and/ or flavoring additives. These flavonols improve taste and sweetness but are not substitutes for sweeteners and flavorings as they do not have taste and are a little astringent. Some authors have pointed out that their role is to make the receptors in the mouth more sensitive to sweeteners, thus lowering the levels of the sweeteners and flavorings used (Kashket 1990; Ochoa M and Ayala A 2004).

1.2.2.2 Tannins

Tannins are classified into two groups: non-hydrolyzable or condensed tannins and hydrolyzable tannins (**Figure 2**). The condensed tannins are also called proanthocyanins, and are bound to the flavanols (Kashket 1990). The content of condensed tannins in strawberries is approximately 54–163 mg/100 g raw strawberry (Basu et al. 2014). In strawberries, the most relevant condensed tannins are procyanidins from catechin and its polymers. Condensed tannins are commonly found in the pulp of strawberries and achenes (Giampieri et al. 2012). Due to the variety of physiological activities, they have been reported to possess, directly and indirectly, antioxidant, antimicrobial, anti-allergic and antihypertensive properties, as well as to inhibit the activities of some enzymes and physiological receptors (Santos-Buelga and Scalbert 2000).

The most common hydrolyzable tannins in strawberries are ellagitannins, specifically, sanguiin H-6 and ellagic acid (Aaby et al. 2005, 2007; Klopotek et al. 2005; Giampieri et al. 2012). The content of ellagitannins in strawberries is approximately 10–23 mg/100 g raw strawberry (Basu et al. 2014). Ellagic acid is an ellagitannin present in the secondary metabolism of vegetables, its main characteristic is its antioxidant, antimicrobial, antimutagenic, anticarcinogenic and antiviral capacity (Basu et al. 2014). The content of ellagic acid in strawberries is approximately 1–2 mg/100 g raw strawberry (Basu et al. 2014). Due to the phenol nature of ellagic acid, this compound tends to react by forming complexes with other molecules of proteins, alkaloids, and polysaccharides, so that it is usually found as ellagitannins esterified with glucose, because of this it is difficult to find it free (da Silva Pinto et al. 2008). The properties of ellagic acid are also exploited in the food industry, so it is used in the manufacture of nutraceutical drinks and food supplements. Likewise, the application of ellagic acid for food preservation is of great impact for the perishable food industry, using its antioxidant activity for microorganisms inhibition (Saucedo-Pompa et al. 2009).

1.2.2.3 Phenol acids

Strawberries contain a variety of phenol acids which are presented as derivatives of the hydroxycinnamic acid, such as caffeic acid, and hydroxybenzoic acids such as gallic acid (Giampieri et al. 2012). The content of phenol acids in strawberries is approximately 0.8–6.7 mg/100 g raw strawberry (Basu et al. 2014). The major hydroxycinnamic acid in strawberries is p-coumaroylhexose, but ferulic acid and caffeic acid glycosides have also been identified in strawberries (Aaby et al. 2005, 2007). Hydroxycinnamic acid derivatives are responsible for the bitter taste of the strawberry, and it is used in the manufacture of creams (Porras-Loaiza and López-Malo 2009; Joseph and DiNardo 2017). The primary derivative of hydroxybenzoic acid is p-hydroxybenzoic glycoside (Klopotek et al. 2005). The p-hydroxybenzoic glycoside is widely used in the synthesis of organic compounds and their esters, known as parabens, which are used as preservatives in cosmetics (Ma et al. 2016).

1.3. Extraction and purification of bioactive compounds in strawberry extrudate

1.3.1 Extraction techniques

1.3.1.1 Solvents extraction

The extraction with solvents is a technique to isolate a substance from a solid or liquid mixture. This technique is currently used in combination with other techniques such as microwaves and ultrasound since the solvent only extracts soluble compounds. Due to the strawberry extrudate nature, the solid–liquid extraction can be carried out with a Soxhlet extractor, which is one of the most commonly used conventional extraction techniques (Azmir et al. 2013). The extraction efficiency depends mainly on the choice of solvents (Cowan 1999). The polar character of the bioactive compounds allows their solubility in various solvents, such as water, alcohols, and acetone (Zapata et al. 2014).

Recently, numerous studies have explored the extraction of bioactive compounds using deep eutectic solvents from various groups of natural sources (Zainal-Abidin et al. 2017). The formation of eutectic solvents is the result of the complexation of a halide salt, which acts as a hydrogen bond receptor, and a hydrogen bond donor (Zainal-Abidin et al. 2017). Some eutectic solvents have been developed from the combination of primary metabolites and bio-renewable starting materials, e.g., sugars, alcohols, amino acids and organic acids (Zainal-Abidin et al. 2017). Eutectic solvents produce less adverse effects on the environment, allowing to replace conventional chemical methods (Zainal-Abidin et al. 2017).

There is a long variety of studies on solvent extraction focusing on the extraction of bioactive compounds. An evaluation of the effect of different solvents and acids in the extraction of anthocyanins from strawberry fruits concluded that acetone provided an efficient and reproducible extraction, avoiding problems with pectins and allowing the concentration of the sample at low temperature (Garcia-Viguera et al. 1998). In another study, it was observed that the acetone/acetic acid mixture (99:1, v/v) reached good results for the qualitative and quantitative evaluation of phenol compounds present in strawberries (Kajdžanoska et al. 2011).

1.3.1.2 Hydrothermal extraction

Hydrothermal extraction is a process in which the matter is treated by adding hot water or water vapor (Azmir et al. 2013). Steam explosion is another kind of hydrothermal treatment where the matter is treated with saturated water vapor at high pressure followed by rapid depressurization (Rincón et al. 2016). The disadvantage of using hydrothermal treatments is that they affect thermosensitive compounds and might form undesirable compounds (Struck et al. 2016).

Hydrothermal extraction at low temperature, i.e., ranging between 50 and 90°C, mainly induces the de-flocculation of macromolecules (Jain et al. 2015). Hydrothermal extraction at medium temperature, i.e., ranging between 150 and 180°C, solubilizes cellulosic and hemicellulose biomass (Hendriks and Zeeman 2009). The steam explosion treatment, with temperature ranging between 180 and 260°C and increase in pressure of 0.69–4.83 MPa, it is able to solubilize lignocellulose biomass (Rincón et al. 2016).

Several studies confirm the successful extraction of bioactive compounds by these hydrothermal extractions (Mrabet et al. 2016; Serrano et al. 2017). Extraction of bioactive compounds in strawberry extrudate has been studied by applying hydrothermal treatments in the range of 90–200°C (Rodríguez-Gutiérrez et al. 2019). Hydrothermal treatment at 150°C for 60 minutes was the most efficient process based on the solubilization of sugars and phenols as well as the antioxidant capacity of the liquid phase produced (Rodríguez-Gutiérrez et al. 2019).

1.3.1.3 Microwave assisted extraction

Microwaves are electromagnetic fields in a frequency range of 300 MHz to 30 GHz, which are generally operated at a frequency of 2.45 GHz (Wijngaard et al. 2012). Microwaves can access biological matrices and interact with polar molecules, such as water, which vibrate or rotate by the effect of microwaves and generate heat and can enhance the processes of extraction of bioactive compounds (Wijngaard et al. 2012; Flores 2017). Microwave assisted extraction has been successfully applied in anthocyanin extraction processes in grape skins (Liazid et al. 2011), the recovery of pectins from press residues of various berries, i.e., red and black currant, raspberry and elderberry (Bélafi-Bakó et al. 2012) and to extract phenol antioxidants from peanut skins (Ballard et al. 2010).

1.3.1.4 High hydrostatic pressure extraction

High hydrostatic pressure extraction is a method that works at high pressures ranging from 100 to 1000 MPa. These high pressures cause cell deformation, cell membrane damage, protein denaturation, deprotonation of charged groups, and the breakdown of bonds, making bioactive compounds more accessible for extraction (Shouqin et al. 2005). High hydrostatic pressure extraction is considered to be a faster and more efficient technique than other conventional extraction methods (Corrales et al. 2008; Patras et al. 2009). In addition, high hydrostatic pressure extraction has the advantage of not increasing the temperature during the processing time, so it would be an ideal method to extract thermosensitive compounds.

Several high hydrostatic pressure extraction studies have been carried out with strawberries for the extraction of bioactive compounds. The impact of high hydrostatic pressure extraction on total strawberry puree phenols was observed by Patras et al., (2009), which reported that the amount of total phenols increases as the pressure in high hydrostatic pressure extraction increases (Patras et al. 2009). In another study, the change in kaempferol, and quercetin quantity in strawberries pulps were tested at different pressures and for different processing times (Cao et al. 2011). According to this study, the change in the amount of kaempferol was not very significant and the amount of quercetin increased with increasing pressure (Cao et al. 2011). Another study showed that the nutritional and sensory qualities of strawberry puree after high-pressure processing at 500 MPa and 50°C for 15 min were much better than after a heat treatment at 90°C for 15 min (Marszałek et al. 2015).

1.3.1.5 Pulsed electric fields extraction

Pulsed electric fields or high intensity pulsed electric fields consist of a short time electrical treatment, between nanoseconds to milliseconds, in which the material located between two electrodes is exposed to a strong electric pulse of intensity field of 100 to 300 V/cm Pulsed Electric Fields or 20 to 80 kV/cm high intensity pulsed electric field, the operation parameters being the duration and number of pulses (Parniakov et al. 2013; Yan et al. 2017). Pulsed electric fields can produce the electrical rupture of the cell membranes producing the formation of pores, what is known as electroporation (Neuman et al. 1982; Parniakov et al. 2013). Pore formation improves cell permeability allowing the recovery of bioactive compounds (Angersbach et al. 2000). Compared to other non-thermal treatments such as the high hydrostatic pressure extraction method, pulsed electric field extraction methods require a much shorter processing time, higher extraction efficiency and these techniques can be easily applied in continuous operation (Yan et al. 2017). Therefore, pulsed electric fields is a promising technique for different applications in the food industry because they can improve extraction capacity and recovery of nutritionally valuable compounds as well as the bioavailability of micronutrients and compounds in a wide range of foods (Yan et al. 2017).

Several studies on the extraction of antioxidant compounds in agro-foods show enhanced yields with pulsed electric fields. For instance, a comparison study between a heat treatment at 90°C for 60 or 30 seconds and high intensity pulsed electric field in strawberries juice, showed that strawberry juice treated with high intensity pulsed electric field maintained greater amount of phenol acids and total anthocyanins than thermally treated juices (Soliva-fortuny and Martín-belloso 2008; Odriozola-Serrano et al. 2009). Likewise, the recovery of phenols from the shell of the pomegranate by pulsed electric field has been assayed, resulting in a similar antioxidant extraction yields and an energy saving of 50 % compared to an ultrasound extraction technique (Pan et al. 2012).

1.3.1.6 Extraction techniques comparison

After reviewing the different extraction techniques that have been applied to the strawberry and strawberry extrudate, a summary describing their most interesting aspects is shown in **Table 1**. The aspects that have been compared are: the specificity of the extraction techniques with the bioactive compounds, the possibility of combining with other extraction techniques, the ability to release bioactive compounds, the potential degradation of bioactive compounds, possibility of intracellular attack, bonds breakage and whether the technique has a high operational and investment cost. The choice of the best technique for the strawberry extrudate is a tailor-made solution for each situation that will depend on the investment capacity, target compounds to be recovered or the required extraction yield.

Table 1.1. Summary Table of Characteristics for Comparing Extraction Techniques.

Extraction technique	Specificity	Possibility of combination	Ability to release compounds	Degradation of bioactive compounds	Intracellular attack	Breaks of bonds	High cost
Solvents extraction	x	x					
Hydrothermal extraction		x	x	x		x	
Microwave assisted extraction		x	x	x	x	x	
High hydrostatic pressure extraction		x	x	x	x	x	x
Pulsed electric fields extraction		x	x	x	x	x	

1.3.2 Purification techniques of bioactive compounds

1.3.2.1 Adsorbents

There are many studies that show the properties of adsorbents to separate, concentrate and purify various compounds (Qiu et al. 2007; Soto et al. 2011). Functionality, porosity, irregularities, surface area, tightly bonded impurities, internal porous structure, particle size, ionic strength, pH, and temperature all influence physical adsorption (Soto et al. 2011). The temperature influences the adsorption in two ways, increasing the transport speed through the outer boundary layer and inside the pores due to the decrease in the viscosity of the solution, and changing the capacity of the adsorbent. However, high temperatures can promote irreversible interactions (Qiu et al. 2007). Another important parameter for purification with adsorbents is pH. For example, at acid pH, the adsorption of phenol compounds by different adsorbents increases because the phenols are not dissociated and dispersion interactions predominate (Soto et al. 2011). At alkaline pH, the adsorption decreases due to the dissociation of hydroxyl groups and carboxyl groups (Soto et al. 2011). There are many types of adsorbents such as activated carbons, mineral adsorbents, synthetic polymeric adsorbents, ion exchange resins, lignin and lignocellulosic materials, adsorbents based on polysaccharides and others (Soto et al. 2011). Among the available adsorbents Amberlite XAD adsorbents are widely used in the concentration of phenol compounds (Ahmad et al. 2015). Zhang et al. (2008) reported the isolation and structural characterization of 10 phenol compounds from strawberry extracts using a combination of Amberlite XAD-16 and C18 columns, HPLC-UV, and nuclear magnetic resonance spectroscopy methods.

1.3.2.2 Countercurrent-chromatography

Countercurrent chromatography is a technique widely used in the purification of natural products (Valls et al. 2009). Countercurrent chromatography is a liquid–liquid partition chromatography process in which both the mobile phase and the stationary phase are liquid (Valls et al. 2009). The main advantage of countercurrent chromatography, when compared to equivalent techniques such as low pressure liquid chromatography, is that there are no adsorption losses in the stationary phase (Valls et al. 2009). The range of selectivity offered by chromatographic resins is equivalent to the range of selectivity offered by the different solvent systems (Valls et al. 2009).

Several studies have shown the importance of countercurrent chromatography for the purification of bioactive compounds from strawberry. The compound 2,5-dimethyl-4-hydroxy-3[2H]-furanone 6’O-malonyl-β-d-glucopyranoside was isolated from a strawberry glycosidic extract (*Fragaria × ananassa, cv. Senga Sengana*) by countercurrent chromatography (Roscher et al. 1996). Peonidin-3-glucoside and malvidin-3-glucoside were obtained from grapes in a single step, while in a second step, cyanidin-3-glucoside was isolated (Renault et al. 1997). In another research, the separation of anthocyanin monomers of high purity from mulberry fruits was developed (Chen et al. 2017).

1.3.2.3 Two-phase aqueous system

Two-phase aqueous system is a liquid–liquid fractionation technique that is usually formed by mixing two polymers in aqueous media, for example, polyethylene glycol and dextran or maltodextrin, or by a polymer and a salt, such as polyethylene glycol and salts of phosphates, citrates, or sulphates (Dutra Molino et al. 2013; Feng et al. 2015; Iqbal et al. 2016). This method has advantages over other purification techniques due to a comparatively low consumption of energy and time, as well as the possibility to be designed for a continuous operation. Moreover, two-phase aqueous systems are effective for many types of substances, especially for the concentration and purification of bioactive compounds (Dutra Molino et al. 2013; Iqbal et al. 2016). Several studies have demonstrated the suitability of this technique for the purification of bioactive compounds such as phenol compounds from fig fruits (Ficus carica L.) (Feng et al. 2015), or the purification of gallic acid from natural matrices with ionic liquids (Cláudio et al. 2012). Furthermore, two-phase aqueous system has been applied for the purification of phenol compounds from a model solution of gallic acid and three real samples of red and white wine, and orange juice in combination with macro and micro extractors (Tušek et al. 2017). Phenol compounds have been also extracted from Aronia melanocarpa berries, using ultrasound-assisted extraction in combination with the two-phase aqueous system (Xu et al. 2017).

1.4. Stabilization of biomass by obtaining bioenergy and bioproducts

1.4.1 Biogas production

Anaerobic digestion is a microbiological process, in absence of oxygen, where organic matter is progressively degraded by a heterogeneous bacterial population to methane (55–70 %) and carbon dioxide (30–45 %) (Monge et al. 2013). Anaerobic digestion presents some fundamental advantages such as the possibility of working at high rates of organic load, and the produced methane can be used as an energy source due to its heating value (35,793 kJ/m^3, at 1 atm, 0°C), which equals to 1 kg of raw coal or 0.76 kg of standard coal (Serrano et al. 2013; Fermoso et al. 2018). The use of biogas for energy supply reduces deforestation, soil erosion and environmental pollution (Pérez et al. 2014; Bożym et al. 2015). Also, it can improve the energy efficiency of various production processes due to the energetic contribution that provides (Bożym et al. 2015). In addition, a wet waste called digestate, which is a mixture of partially degraded organic matter, microbial biomass, and inorganic compounds, is produced during biomethanization and could be used as a base for fertilizers or organic amendments (Serrano et al. 2014a; Bożym et al. 2015).

Several studies on anaerobic digestion of strawberries extrudate have been carried out. The results of one these studies reveal that strawberries extrudates have a high level of anaerobic biodegradability (90 % in VS, (total volatile solids)) and that a substantial amount of methane can be obtained in this way (312 mL CH_4 STP/g added VS) (STP: standard temperature and pressure conditions, i.e., 0°C, 1 atm) at an organic loading rate range of 2.04 to 3.51 kg VS/m^3·d (Siles et al. 2013). In another study of anaerobic digestion of strawberry waste from supermarkets, using an organic loading rate of 0.55–4.4 (g/L·d), the experimental biogas and methane yields were 0.588 and 0.231 L/g, respectively (Arhoun et al. 2017). It has been observed that sometimes it is necessary to co-digest strawberry extrudate with a substrate that provides alkalinity, such as sewage sludge (Serrano et al. 2014b, 2015). Co-digestion studies of strawberry extrudate with other substrates such as fish waste (Serrano et al. 2013) and glycerol (Serrano et al. 2014a) have also been studied. Anaerobic digestion of strawberry extrudate is a promising technique but it should be further studied since low alkalinity of the extrudate together with formation of inhibitory compounds caused by the extraction process could negatively affect the digestion process.

1.4.2 Compost production

Composting has been proposed for a long time as a quite cheap option for agricultural waste management (Gutiérrez et al. 2017). Composting has also been proposed as a post-treatment for the produced digestate after anaerobic digestion (Vaneeckhaute et al. 2017). Composting is the biooxidative conversion of organic waste into an organic amendment. According to Gutiérrez et al. (2017), the cost of composting varies in a wide range from $40 to $500 per throughput ton depending on the technology. Composting costs vary widely depending on the type of operation, which ranges from the most simple ones, such as opening windrows, to more complex procedures like in-vessel aerobic composting that allows smell emissions to be controlled and prevents environmental pollution (Gutiérrez et al. 2017). The great disadvantage is that a considerable amount of offensive odors can be emitted during the process due to the generation of volatile organic compounds (Toledo et al. 2017). Other disadvantages are the long process time and the necessity of a proper monitoring (Parthiba Karthikeyan et al. 2018). Co-composting of a waste mixture containing strawberry extrudate, fish waste, sewage sludge, and bulking agent has been successfully proven (Gutiérrez et al. 2017; Toledo et al. 2017).

1.4.3 Bioethanol production

Bioethanol is one of the most produced alcohols from the fermentation of sugars found in fruits and vegetables (Dias et al. 2009; Goh et al. 2010; Gupta and Verma 2015; Guerrero et al. 2018). Theoretically, any organic product with a high content of sugars and starch, such as strawberry extrudate, may be susceptible to obtaining bioethanol (Goh et al. 2010). Inedible sources from the strawberry extrudate such as lignocellulosic biomass, which mainly comprises cellulose, hemicellulose, and lignin, can be hydrolyzed to produce a mixture of pentoses and hexoses that can be transformed into bioethanol (Tan et al. 2008). Bioethanol from agro-wastes, such as strawberry extrudate, could be a promising technology that involves four processes, pre-treatment, enzymatic hydrolysis, fermentation and distillation, this final step is crucial for the process to be economically viable on a commercial scale due to high energy consumption in the form of steam to increase the yield of bioethanol production when lignocellulose materials are used as raw material (Gupta and Verma 2015). These processes have several challenges and limitations, such as the efficient pre-treatment process to eliminate lignin from the lignocellulosic agro-wastes. The proper pre-treatment process can increase the concentrations of fermentable sugars after enzymatic hydrolysis, thus improving the efficiency of the entire process (Guerrero et al. 2018).

1.4.4 Bioplastics production

Fossil fuel depletion, global warming, and problems of pollution of the environment that provoke plastics in its life cycle are encouraging the development of biodegradable plastics (Heredia-Guerrero and Athanassiou 2016; Heredia-Guerrero et al. 2017). Agro-waste are usually rich in many useful substances such as lipids, polysaccharides, and aromatics, which could be used for the manufacture of biodegradable polymeric materials. Bioplastics already play an important role in the sectors of packaging, agriculture, consumer electronics, and motoring, but still have a very low share in the total production of plastics. Currently, about 1 % of the annual tons of plastic are bioplastics (Rujnić-Sokele and Pilipović 2017). Examples of such bioplastics are exopolysaccharides, polycaprolactone, polybutylene succinate, polybutylene adipate terephthalate, polyhydroxyalkanoates, or polyhydroxybutyrates (Yaradoddi et al. 2016; Rujnić-Sokele and Pilipović 2017). For obtaining bioplastics from agro-waste, the waste must be treated to extract or isolate specific macromolecules, such as cellulose, lignin, suberin, starch, or monomers, such as vegetable oils, tannins and terpenes (Gandini et al. 2016; Heredia-Guerrero et al. 2017). A study conducted on the production of bioplastics from Murta fruit extract, that is a native Chilean berry, showed the feasibility of using berries for bioplastic production (López de Dicastillo et al. 2016).

1.4.5 Biochar production

Biochar is the solid carbonaceous residue produced through organic waste and used as a soil improver (Oliveira et al. 2017; de la Rosa et al. 2018). Biochar is produced through several types of methods such as pyrolysis, torrefaction, or hydrothermal carbonization (Oliveira et al. 2017; de la Rosa et al. 2018).

There are no studies reported in the literature dealing with the production of biochar from strawberry extrudate. However, the above-mentioned techniques (pyrolysis, torrefaction, and hydrothermal carbonization) could be potentially applied for this substrate. Several studies have been carried out on the hydrothermal carbonization of other agro-waste, such as olive cuttings and olive pulp (Volpe and Fiori 2017); grape marc (Basso et al. 2014); olive mill waste, canned artichoke and orange waste (Benavente et al. 2015).

1.5. Inverse vulcanization as an option for the exploitation of bioactive compounds

An Earth-abundant element that has been mined for thousands of years is elemental sulfur (S_8). Currently, sulfur is the tenth most abundant element on Earth and it is valuable for the scientific community as achemical reagent with important uses in industry, agriculture, or material science (Boyd 2016). Until the 20th century, the most common source of sulfur was the soil surrounding volcanoes, but nowadays, starting with the development of the crude oil refining industry, the production of elemental sulfur (S_8) is achieved by removing the H_2S gas from crude oil. Around 50 million tons of sulfur are yearly obtained as by-product from the refining gas and petroleum industry (Zharylkassyn et al. 2021). Currently, sulfur has several uses for the industry, but there is still a net excess of sulfur that offers few economic uses, thus finding a new large-scale use for this sulfur would be a valuable development. The storage of sulfur that is formed as a by-product in the crude oil purification process represents an important problem due to its high polluting capacity and its low selling price. However, its use may be an opportunity as an economical starting material in the synthesis of other high added value compounds.

Sulfur has some chemical inertness, exhibiting limited solubility in many organic solvents, except for poor solubility in some aromatics such as toluene, carbon disulfide and pyridine (Griebel et al. 2016). All of this makes it hard to synthesize polymers directly from sulfur, under slight reaction conditions. The main limitation being the instability of polysulfides produced by ring opening polymerization (ROP) (**Figure 1.3**) (Griebel et al. 2016). The orthorhombic α-S_8 is the most stable form, where yellow crystals at a temperature of 95 ^{o}C undergo a change to the monoclinic form β-S_8 by changing phase. By continuing to heat molten sulfur above 159 ^{o}C, homolysis of the S–S bond provides thiyl radicals that attack and open the ring of another S_8 molecule. Polymerization is propagated by repeated ring opening and the formation of new S-S bonds between S_8 and the growing polysulfide chain. These high molecular weight polymers formed by ROP are chemically unstable at temperatures above the temperature of the start of polymerization (Tf = 159 °C), since terminal sulfur radicals again promote depolymerization to monomeric sulfur, or even to other cyclic species with S_n ($n \neq 8$), where the voltage of the ring makes them open easily. The use of sulfur as an additive has been known for a long time and the vulcanization processes of rubbers and other polymers of the product obtained thermosetting materials. Chung et al., (2013) developed the revolutionary inverse vulcanization concept that efficiently uses S_8 as a reaction medium and as copolymer with other compounds bearing double bonds, not as an additive in polymerization processes. Polymers with a very high sulfur content (50-90 % sulfur by mass) can be obtained through inverse vulcanization stable (**Figure 1.3**). The use of this new type of copolymers has opened a field to the study of new materials with very useful chemical and optical properties since they have been used in such important fields as materials science, polymer technology, adhesives, electrochemistry, or water treatment. Since it depends on the nature of the bond between the copolymer, the specific surface of the material formed can be modified, as well as its physical-chemistry properties.

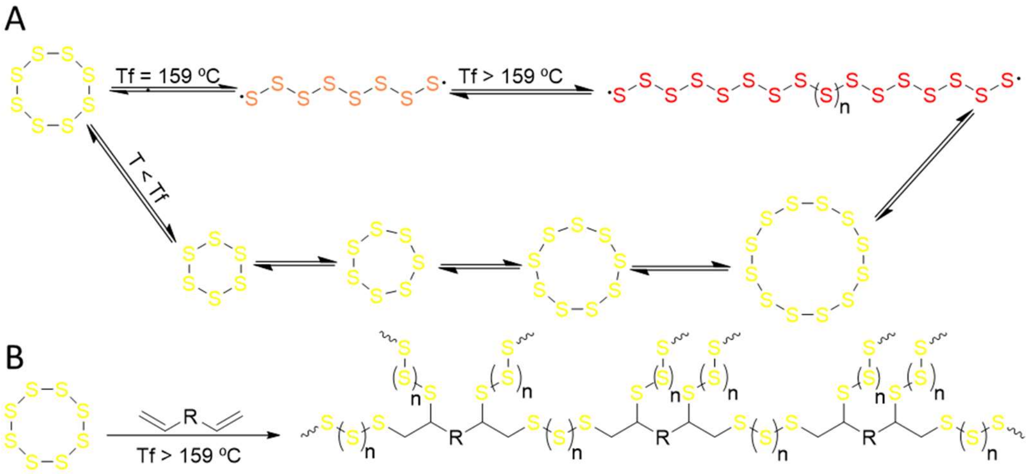

Figure 1.3. (A) Homolytic ring opening polymerization (ROP) of sulfur S_8. (B) Inverse vulcanization reaction.

The properties of the polymers could be controlled by the selection of the polymerization reaction conditions and the use of suitable copolymer, obtaining a wide variation of properties. Vegetable oils are the copolymer more researched, due to properties that can range from highly cross-linked thermosets to linear thermoplastics, making them applicable in a wide range of materials for various uses (Adekunle 2015; Abbasi et al. 2019). Due to these properties, vegetable oils have been made new raw materials for preparing bio-based polymers with many applications such as medical devices and polymeric compounds (Abbasi et al. 2019). Other copolymers could be compounds from strawberry extrudate, which is the source of a great variety of bioactive compounds, as mentioned previously. These bioactive compounds can be used as copolymers for the inverse vulcanization process. A very recent example of the use of an agro-waste as a source of a copolymer compound, such as limonene has been reported in the literature for use as a copolymer in the inverse vulcanization process (Crockett et al. 2016). The use of copolymers of natural origin that come from residues of the agro-food industry would lead to obtaining polymeric material that offers a high degree of biodegradability, antioxidant power, and antimicrobial properties (Griebel et al. 2016; Smith et al. 2020; Guo et al. 2021). In addition, getting polymers that are biodegradable or compostable is crucial today to try to solve the problems inherent in their elimination. It would be very useful to obtain polymers that after their useful life cycle are susceptible to rapid degradation. This is a great challenge because, excluding extreme examples such as polar or desert climates, ambient temperature and humidity are usually not very different vectors to the operating range that most polymers are subjected to in routine uses in different applications.

The potential uses of these new materials can be found in the examples recently described in the literature. Different studies have been carried out on inverse vulcanization with vegetable oils for the development of diverse technology uses. Sulfur copolymers obtained by inverse vulcanization of elemental sulfur and vegetable oils such as sunflower, linseed, and olive were applyed in the development of lithium-sulfur battery cathodes, which demonstrated a remarkable initial specific capacity (Gomez et al. 2016; Hoefling et al. 2017). In another study, soybean oil was chosen to react with elemental sulfur to be investigated as a source of sulfur for plants, as a fertilizer, with improved oxidation capacity (Valle et al. 2019). Sulfur-based polymers obtained from the inverse vulcanization of sulfur with canola oil and recycled cooking oils to produce an adsorbent for mercury capture were also investigated. These adsorbents showed a very high affinity for different types of mercury in liquid and vapor form, in addition to inorganic and organic mercury compounds (Worthington et al. 2017b, a, 2018; Parker et al. 2018).

1.6 Proposal for an experimental study on strawberry extrudate

Taking into account that a good valorization of the strawberry extrudate can be obtained by different processes, an experimental study is proposed after the evaluation of the different techniques previously described techniques (**Figure 1.4**).

Strawberry extrudate may have a composition similar to fresh fruit, so it is very interesting to recover the bioactive compounds contained in this waste. The bioactive compounds of the strawberry could have a great interest for the pharmaceutical and food industries, or as proposed in the study for the synthesis of polymers by inverse vulcanization.

One of the options studied and with great potential, for the recovery of bioactive compounds from strawberry extrudate is the hydrothermal treatment. The hydrothermal treatment could solubilize the bioactive compounds of interest in a liquid phase, this liquid phase being easy to handle for the time of purification of the bioactive compounds of interest. In addition, this method allows for temperature selection to optimize the process, this being interesting for the feasibility and suitability of the process. For the process of purification of bioactive compounds, it is observed that most studies focus on sugars and phenol compounds, as they are beneficial compounds for health with great antioxidant power. One of the simplest and most economical processes is the adsorption with ion exchange resins such as Amberlite XAD resins. A study of the bioactive compounds obtained after hydrothermal treatment is also proposed, for its application in the use of copolymers with sulfur and/or vegetable oils.

Finally, once the bioactive compounds of interest have been obtained, the solid and the liquid phases that remain after hydrothermal treatment with a large amount of organic matter, must be stabilized. One of the methods studied for this proposed is the anaerobic digestion, which might be an interesting option to recover energy from strawberry extrudate, closing the production loop. With an anaerobic digestion process, the thermal energy necessary for the hydrothermal treatments could be obtained and, in addition, the two phases (solid and liquid) obtained after the hydrothermal treatment and the recovery of the bioactive compounds can be stabilized (**Figure 1.4**).

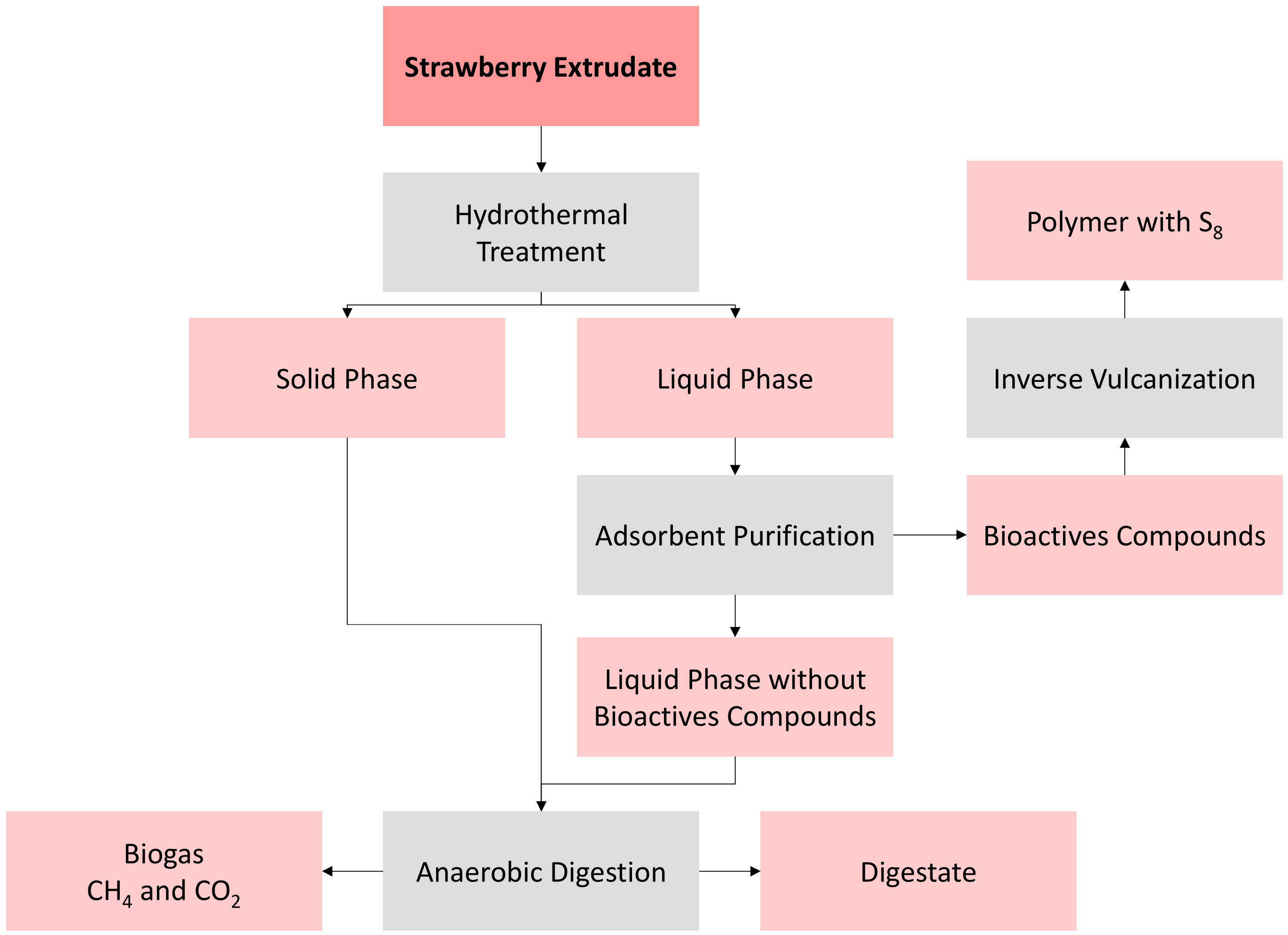

Figure 1.4. General scheme of the experiment performed throughout the PhD thesis work

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2 Hydrothermally-treated strawberry extrudate: A rich source of antioxidant phenols and sugars

Abstract

Strawberries have antioxidant, cardiovascular, and antiproliferative properties. The agro-industrial production of strawberry concentrate generates a food waste after extrudation that is usually landfilled. This strawberry extrudate is a rich source of valuable bioactive compounds such as phenols and sugars. In the present study, industrial hydrothermal treatments currently in use for the valorization of other agricultural wastes were determined to be suitable for the treatment of strawberry extrudate. Hydrothermal treatment conditions in the range of 90 °C to 200 °C were studied. Hydrothermal treatment at 150 °C for 60 min without acid addition was the most efficient process based on the solubilization of sugars and phenols as well as the antioxidant capacity of the liquid phase produced. Instead of sending this residual fraction to landfill, such treatment would permit the use of strawberry extrudate as a source for the recovery of valuable bioactive compounds.

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2.1. Introduction

The global strawberry industry produced 11 million tons of strawberries in 2014. The United States is the world's major producer with 12.2 % of world production, followed by Mexico, Turkey, and Spain (FAO 2017). The strawberry is a popular seasonal fruit due to its unique flavor and aroma. The strawberry is considered a functional food that offers multiple health benefits, including antioxidant, cardiovascular, antihypertensive, and antiproliferative effects (Basu et al. 2014). These health effects are due to strawberry's unique combination of nutrients, phytochemicals, and fibers, which play a synergistic role in characterizing strawberries as a functional food (Basu et al. 2014). Moreover, strawberries are an important source of B-group vitamins, vitamin C, vitamin E, potassium, folic acid, carotenoids, and specific flavonoids, such as pelargonidin, quercetin and catechin (Giampieri et al. 2012) or the tiliroside that is a glycosidic flavonoid and possesses anti-inflammatory, antioxidant, anticarcinogenic and hepatoprotective activities (Goto et al. 2012).

The high antioxidant capacity of strawberries is mainly due to the presence of ascorbic acid, ellagitannins and anthocyanins (Basu et al. 2014). Anthocyanins give the fruit its characteristic red color. Anthocyanins present in the strawberry have been investigated and identified as cyanidin and pelargonidin glycosides (Cerezo et al. 2010), and pelargonidin-3-glucoside is the main anthocyanin in the strawberry (Cerezo et al. 2010). Other phenol compounds, such as glucosides and glucuronides of quercetin and kaempferol, are also present (Cerezo et al. 2010; Cruz-atonio et al. 2010). There is particular interest in determining strawberries' ellagic acid content due to its possible chemopreventive effects (da Silva Pinto et al. 2008). This compound may exist in a free form, as a glycoside, or bound as glucose esterified ellagitannins, like agrimonin as an ellagitannin dimer (Maas et al. 1991). It is also remarkable the content of ellagitannins with antioxidant and cancer chemopreventive activities that might contribute to health benefits in humans (Cerdá et al. 2005). Other important group of phenols with bioactive properties are the tannins, divided into condensed tannins (proanthocyanidins) and hydrolyzable tannins (Skrovankova et al. 2015).

In addition to strawberry production and commercialization, the strawberry sector includes the manufacture of derived products. For example, around 21 % of the total production of strawberries is used for the elaboration of products such as yogurt, juices, jams, etc. (Serrano Moral 2015). These products are generally elaborated from a strawberry concentrate. During the industrial process to obtain the strawberry concentrate, strawberries are extruded by several sieves with different mesh sizes. The residual fraction formed of the fibers part and the achenes is retained in the sieves and named strawberry extrudate. Strawberry extrudate represents 7 % of the weight of processed strawberries (Serrano et al. 2014). The strawberry extrudate contains most of the beneficial components found in the whole strawberry. Therefore, an interesting revaluation and management option, instead of sending this residual fraction to landfill as in current practice, would be the recovery of bioactive compounds with a high economic interest from strawberry extrudate. Extracts rich in bioactive compounds could be used in a wide range of novel applications because of their proven health effects on long-term consumption. The phenols present in strawberry, and therefore in the strawberry extrudate, have been widely studied and their remarkable antioxidant properties (Banerjee et al. 2017) make them suitable for use as additives in food formulation to prevent oxidation (Balasundram et al. 2006). Thus, their extraction would not only increase the economic revalorization of this waste but also permit the further degradation of the remaining waste since phenol compounds have been reported as potential inhibitors of downstream digestive bioprocesses (Borja et al. 1997; Chen et al. 2008).

The aim of this study was to evaluate different hydrothermal treatment conditions in order to maximize the recovery of bioactive compounds with a high economic interest from strawberry extrudate. The temperature, pressure, time and addition of reagents for each condition used were chosen based on the industrial hydrothermal treatments widely in use for other agro-industrial products or by-products. An organic extraction with ethanol, commonly used in the food industry, has been used as a control. All the conditions tested in the present study have been previously tested for the recovery of high added valuable compounds from olive oil waste, secondary date varieties, asparagus waste, or cocoa husk (Rubio-Senent et al. 2012; Fuentes-Alventosa et al. 2013; Mrabet et al. 2016; Hernández-Hernández et al. 2018).

The hydrothermal treatment consists of an autohydrolytic that results in the solubilization of the strawberry extrudate (Fernández-Bolaños et al. 2010). Autohydrolysis takes place when acetic acid from acetyl groups is formed because of the high temperatures (Jönsson and Martín 2016). As mentioned, this type of treatment has already been applied to the valorization of other organic waste such as olive mill solid waste (Rubio-Senent et al. 2012). Among the bioactive compounds extracted from olive mill solid waste are phenol compounds, which can be used as preserving agents and/or antioxidants due to their ability to eliminate free radicals and prevent oxidation reactions in food (Banerjee et al. 2017).

In the present study, hydrothermal treatments were tested which was shown to favor the disruption of fibers material (Fernández-Bolaños et al. 2004). Low-temperature hydrothermal treatments mainly induce the de-flocculation of macromolecules with minimal solubilization of the lignocellulosic matter (Jain et al. 2015); whereas high-temperature hydrothermal treatments (150 °C–180 °C) mainly induce the solubilization of the lignocellulosic matter, first the hemicelluloses and shortly after the cellulose and lignin (Hendriks and Zeeman 2009). For steam explosion treatments, after applying a high pressure and temperature, strawberry extrudate is exposed to atmospheric pressure by a quick-opening ball valve, which makes the material undergo an explosive decompression in an expansion chamber. The temperature, pressure, and time of steam explosion treatments range between 160 and 260 °C, 0.69–4.83 MPa, and from several seconds to a few minutes (Fernández-Bolaños et al. 2004). Under steam explosion conditions, hemicellulose is hydrolyzed into its component sugars, and lignin is highly degraded (Fernández-Bolaños et al. 2004).

2.2. Materials and methods

2.2.1. Strawberry extrudate

The strawberry extrudate obtaining from strawberry fruit by twin-screw extruder in cold conditions (below 6 °C) was collected in HUDISA S.A, (Huelva, Spain) in 2017 season and immediately stored at −20 °C to avoid any fermentation or degradation.

2.2.2. Hydrothermal treatments

Low, medium, and high severities of treatment were tested with and without the addition of an acid catalyst (0.5 % glacial acetic acid) by duplicate:

Low-temperature hydrothermal treatments (90 °C–120 °C) were carried out by heating in a laboratory stove and autoclaving at 90 °C and 120 °C, respectively. For the 90 °C treatment, 0.4 kg of strawberry extrudate with 0.74 L of distilled water were introduced into a Pyrex bottle and kept at 90 °C for 90 min in a laboratory stove (J.P. Selecta). For the 120 °C treatment, 0.3 kg of sample with 0.3 L of distilled water were introduced into a Pyrex bottle and kept in an autoclave (Trade Raypa Steam Sterilizer) at 120 °C for 60 min. After each treatment, the wet solid was centrifuged (Pacisa, Milan, Italy) at 7155g for 10 min to separate the solid and liquid phases.

High-temperature hydrothermal treatments were carried out using a steam treatment reactor with 100 L of capacity, which can reach temperatures up to 190 °C and a maximum pressure of 1.2 MPa. Heating of the strawberry extrudate was performed by direct steam injection. Extrudate samples (6 kg) were treated at 150 °C and 170 °C in the reactor for 60 min. After the treatment period, the sample was cooled to 50 °C and then centrifuged at 4700g/1450 rpm (Comteifa, S. L., Barcelona, Spain) to separate the liquid and solid phases.

Steam explosion treatments were performed in a pilot-scale reactor (Nusim, S.A., Madrid, Spain). The reactor is equipped with a stainless steel deposit with 2 L of capacity. The steam explosion reactor was loaded with 250 g of strawberry extrudate which was heated at a temperature of 180 °C–240 °C with high-pressure saturated steam (with a corresponding pressure of 0.78–4.27 MPa) for 2 and 5 min. An electronic computing device controls the time and the temperature in a pre-programmed manner. After each treatment, the wet solid was filtered in a Buchner funnel using Whatman filter paper discs to separate the solid and liquid phases. A concentration of 0.5 % glacial acetic acid was used in each hydrothermal treatment to study the effect of acid addition in the solubilization of phenols and sugars.

2.2.3. Chemicals

The chemicals trifluoroacetic acid (TFA), anthrone, Folin-Ciocalteu's phenol reagent, hydroxymethylfurfural, and 2,2-diphenyl-1-picrylhydrazyl were purchased from Sigma-Aldrich (Madrid, Spain). Sodium bicarbonate (Na_2CO_3), ethanol, and methanol were from Panreac Quimica S.A. (Barcelona, Spain). Concentrated acetic acid was purchased from Fluka (Switzerland). Acetonitrile was of HPLC-grade purity (Romyl, Teknokroma, Barcelona, Spain).

2.2.4. Preparation of liquid and solid phases

The scheme of the extractions is showed in **Figure 2.1**. To extract the soluble compounds from the liquid phase, the liquid phase was centrifuged again to remove solids in suspension and microfiltered with 0.45 µm nylon microfilters.

A technique widely used for composting analysis and based on water extraction (Thompson et al. 2001) was applied to quantify the soluble compounds in the solid phase. To 20 g of solid fraction, 160 g of distilled water was added. After stirring for 24 h, the mix was centrifuged and microfiltered with 0.45 µm nylon microfilters.

2.2.5. Solid phase characterization

The determination of pH, chemical oxygen demand (COD), soluble COD (sCOD), total solids (TS), mineral solids (MS), and total volatile solids (VS) to characterize the solid phase were developed following the recommendations of the Standard Methods of APHA (2012), as previously described (Serrano et al. 2017).

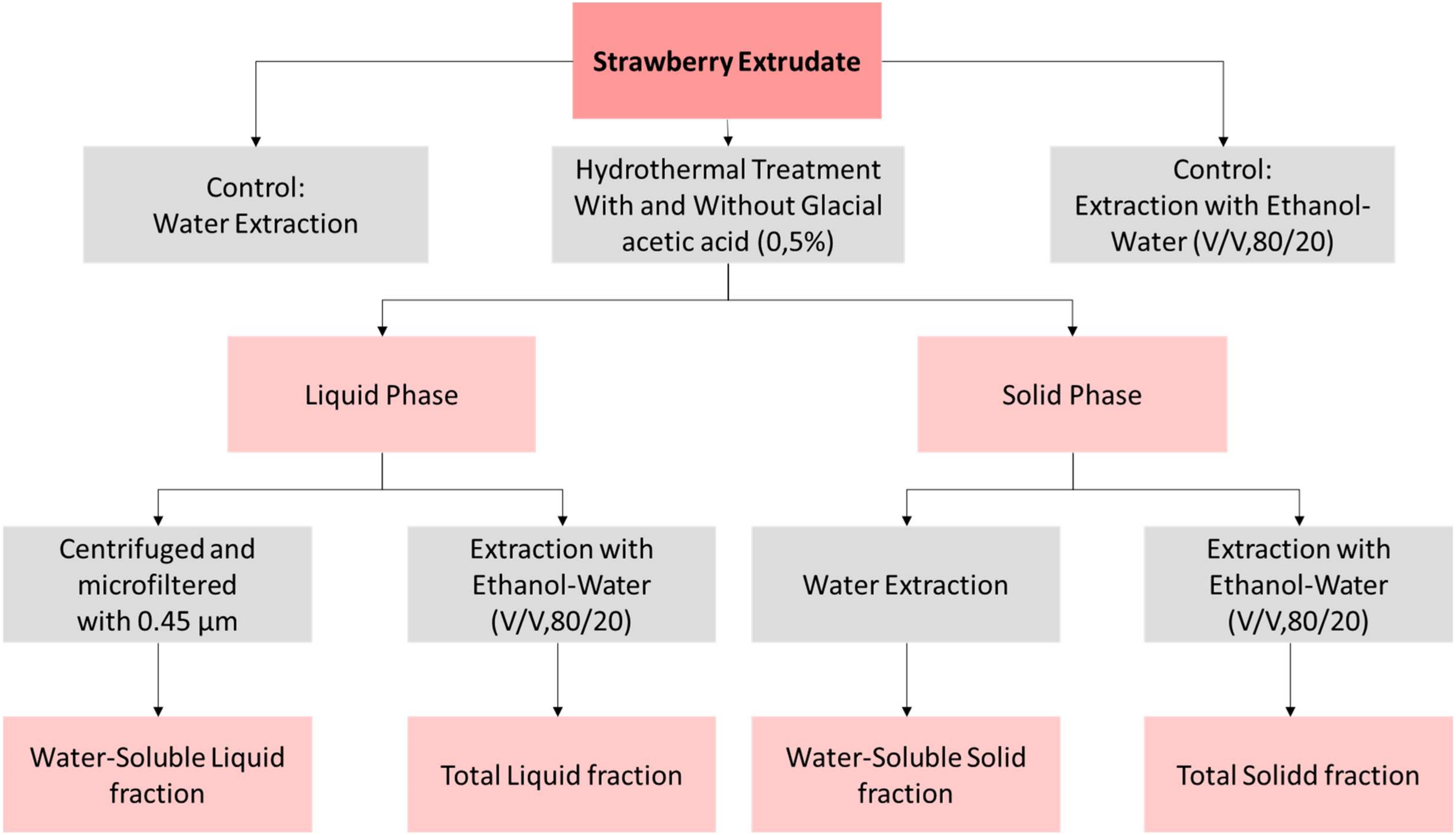


Figure 2.1. Scheme of the solid and liquid extraction using water and ethanol for the phenol compounds, sugars and uronic acid determination.

2.2.6. Analytical methods

2.2.6.1. Hydroxymethylfurfural (HMF)

The identification of HMF was performed by high performance liquid chromatography (Hewlett-Packard model 1100, Palo Alto, CA, USA) equipped with an array detector monitoring at 280 nm and a C18 reverse-phase column (Spherisorb ODS-2; 250 × 4.6 mm i.d. and 5 µm particle size) supplied by Teknokroma (Barcelona, Spain) kept at 25 °C with a C18 guard column. All aliquots of liquors from hydrothermal treatments were filtered through 0.45 µm membranes and injected directly into the HPLC instrument. A flow rate of 1.0 mL/min and an injection volume of 20 µL were used. Separation was achieved using two solvents: solvent A (Milli-Q water, pH 2.5 adjusted with 20 mM TFA) and solvent B (acetonitrile). A linear gradient analysis was used as follows: Starting with 95 % solvent A and 5 % solvent B, this was increased to 25 % solvent B over 30 min. The system was equilibrated between runs for 5 min using the starting mobile phase composition. The identification of HMF was based on the comparison of retention times with those of reference compound and the UV spectrum was recorded in the range of 200–360 nm. Quantification was performed using regression curves in triplicate for four different concentrations ($R^2 \geq 0.99$).

2.2.6.2. Total and water-soluble phenol compound

In order to obtain total phenols, 20 mL of methanol-water (v/v, 80/20) was added to 10 g of the solid or liquid fraction and incubated for 1 h at 70 °C in a water bath then microfiltered with 0.45 µm nylon microfilters. The total phenols and water-soluble content was determined by the Folin-Ciocalteu spectrophotometric method and expressed as grams of gallic acid equivalents (Singleton and Rossi 1965).

2.2.6.3. Total sugars and uronic acids

Total sugars were analyzed by the Anthrone colorimetric method (Witham et al. 1971) using a spectrophotometer (BIO-RAD iMark Microplate Reader, USA). Uronic acids were measured by the m-hydroxydiphenyl method, as described by Blumenkrantz and Asboe-Hansen (1973), and expressed as grams of galacturonic acid per kg of fresh strawberry extrudate.

2.2.6.4. Individual neutral sugars

Individual neutral sugars were analyzed from duplicate samples of solubilized fractions with and without initial TFA hydrolysis prior to reduction, acetylation, and analysis by gas chromatography (GC), using a method described by Lama-Muñoz et al.,(2012).

2.2.7. Antioxidant determinations

2.2.7.1. Antiradical activity: 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The antioxidant activity of each liquid fraction obtained after hydrothermal treatments and the untreated control was determined by the free radical scavenging capacity, using the DPPH method as previously described (Rodríguez et al. 2007). The antioxidant capacity of each liquid fraction was expressed as EC50 (effective concentration, mg/mL), as calculated for each antioxidant from a calibration curve using linear regression.

2.2.7.2. Reducing power

The method described by Rodríguez et al. (2007) was used to determine the reducing power of each liquid fraction after hydrothermal treatment. Briefly, the solutions were treated by L^{-1} $FeCl_3$ in citric acid. The mix was measured in a microplate reader in quadruplicate, including a blank without $FeCl_3$. The microplate was incubated during 20 min at 50 °C, and after a prewarmed dipyridyl solution in trichloroacetic acid was added, read at 490 nm. Reducing power (RP) was expressed as quercetin equivalents (g/L QE) from the equation as determined from linear regression: $RP = 0.2172 \times A490– 0.018$.

2.2.8. Statistical analysis

Results were expressed as mean values ± standard deviations. To assess the differences between samples, a comparison was performed using the Statgraphics Plus program version 2.1. Multivariate analysis of variance (ANOVA) followed by Duncan's comparison test was performed. Results were considered statistically significant for $p < 0.05$.

2.3. Results and discussion

2.3.1. Solubilization of sugars

The addition of acid increased the concentration of solubilized total sugars in the liquid fraction separated after hydrothermal treatments in most cases (**Figure 2.2 A**). The exception was steam explosion treatment for 5 min, which caused a significant degradation of sugars due to the high temperatures used. The best solubilization of total sugars from the liquid fraction was obtained with treatment at 90 °C with acid addition (0.5 % glacial acetic acid), i.e., 65 g/kg SE. The concentration obtained at 90 °C with acid addition indicated that sugars were released easily by the de-flocculation of macromolecules and hemicellulose solubilization. Sugar solubilization from lignocellulosic biomass at 90 °C was also previously observed by Mosier et al. (2005). Interestingly, the concentration of solubilized sugars at 150 °C and 170 °C (53–57 g/kg) were only slightly lower than at 90 °C, indicating that even at high temperatures, sugar solubilization was the main process happening. The solubilized sugars in the liquid fraction could be used as a fermentable source for the production of wine or vinegar (Hornedo-Ortega et al. 2017) or for the production of ethanol or ethanol derivatives.

The total values of acid sugars in the liquid fraction, expressed as grams of uronic acids per kg of strawberry extrudate, were directly proportional to the pectin substances (**Figure 2.2 B**). Pectins are widely used in the food industry as gelling or thickening agents (Chamorro and Mamani 2015). The treatment at 150 °C without acid addition produced the highest yield of acid sugars i.e., 8.9 g/kg. Temperatures above 150 °C seemed to cause not only the solubilization of acid sugars but also their degradation, with the exception of treatment at 200 °C with acid addition for two minutes. Uronic acids are released from hemicellulose at high pressure and temperatures (Jönsson and Martín 2016) like the treatment at 200 °C in which the lower reaction time seems to be crucial to avoid this degradation. Therefore, except in the latter treatment, the addition of acetic acid did not improve the concentration of acid sugars solubilized from the liquid fraction of hydrothermally treated strawberry extrudate.

Next, the composition of individual sugars and their distribution in monosaccharides and oligosaccharides, as well as the HMF concentration, as a degradation product of sugars (hexoses), were quantified for the liquid and solid phases of all hydrothermally-treated samples (**Table 2.1**). HMF is neither present in the SE material nor formed at 90 °C. The maximum concentration of HMF was obtained with treatment at 170 °C for 60 min with acid addition. These HMF values were higher than those obtained using steam explosion, which subjected the sample to higher temperatures but for shorter periods of time. HMF is commonly present in food processing products in which a hydrothermal treatment has been applied. HMF has recently been identified within natural extract with high antioxidant properties (Mrabet et al. 2016) that can be used to prevent the oxidation of edible oils, enhancing the commercial life up to four times for sunflower oil. However, the presence of HMF in the liquid phase is controversial since it has been shown to have an inhibitor activity against microorganisms (Ghasimi et al. 2016), which could complicate the use of further downstream bioprocesses for the utilization of the liquid phase.

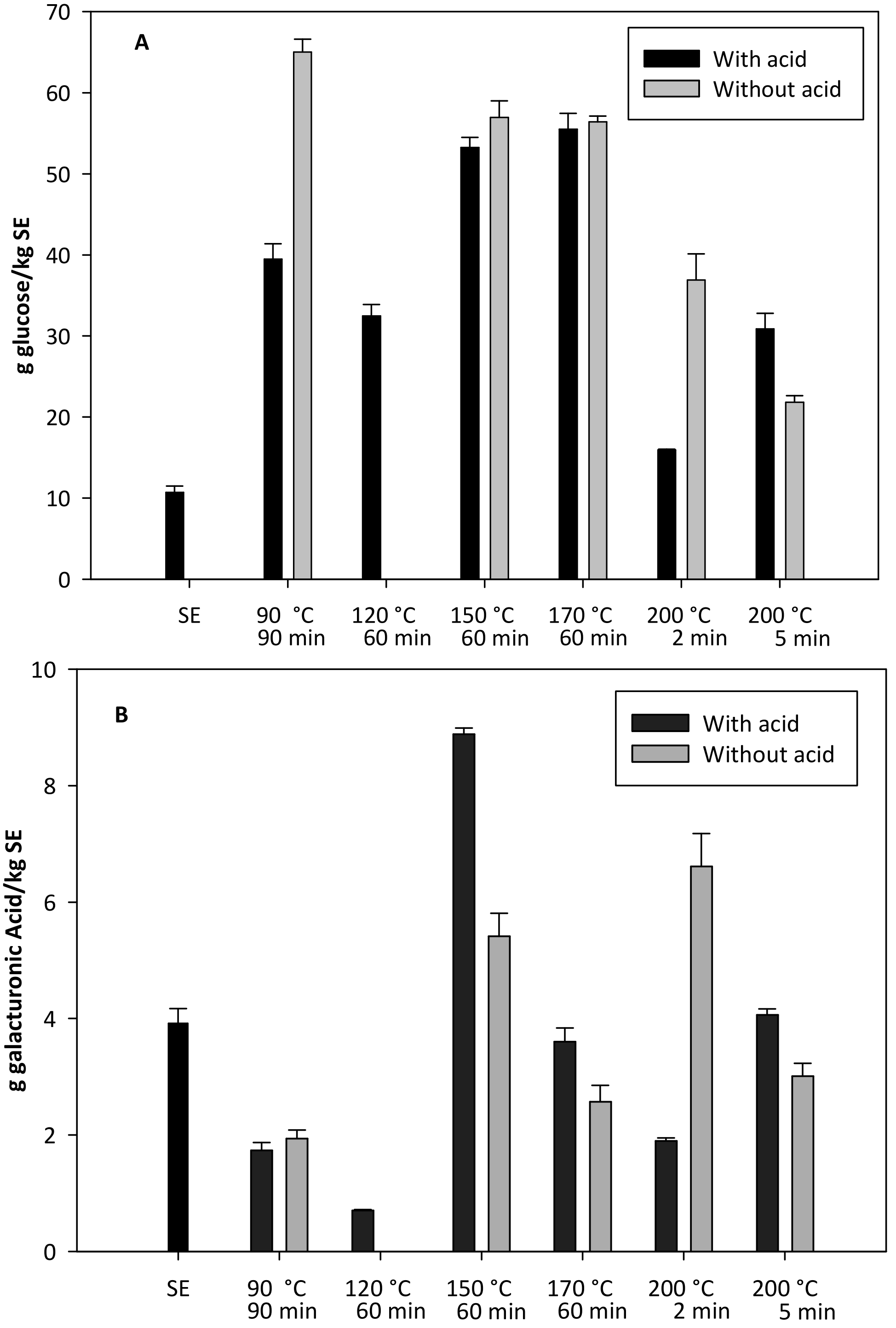


Figure 2.2. Grams of sugars (A) and uronic acids (B), which can be extracted from 1 kg of fresh strawberry extrudate sample using all the hydrothermal treatments tested, with and without acid addition. Untreated strawberry extrudate (SE) was used as control. Means with the same letter were not significantly different, $p < 0.05$.

Table 2.1. Glycoside composition (mg/kg of fresh strawberry extrudate), total monosaccharides (Total MS) and total oligosaccharides (Total OS) and hydroxymethylfurfural (HMF) of the liquid phase (LP) and the solid phase extracted by alcoholic solution (SF) for all the hydrothermally treated samples and the untreated strawberry extrudate (SE) as a control. The analyzed sugars are Rhamnose (Rha), Fucose (Fuc), Arabinose (Ara), Xylose (Xyl), Mannose (Man), Galactose (Gal) and Glucose (Glu). n.d. non detected. Traces: values <0.00. *0.005 meaning value between 0.001 and 0.004. Standard deviations in brackets.

Treatment	Phases	% Acid	Rha	Fuc	Ara	Xyl	Man	Gal	Glu	Total OS	Total MS	HMF
SE			0.02 ±0.00*	n.d.	0.02 ±0.00	0.07 ±0.00	0.90 ±0.03	0.01 ±0.00	2.58 ±0.05	0.46 ±0.00	3.59 ±0.08	n.d.
90 °C. 60 min	LP	0	0.12 ±0.00	n.d.	0.20 ±0.01	0.37 ±0.02	5.72 ±0.20	n.d.	15.45 ±0.77	1.12 ±0.24	21.87 ±0.90	n.d.
		0.5	0.21 ±0.01	n.d.	0.15 ±0.01	0.73 ±0.01	10.36 ±0.33	n.d.	30.16 ±1.42	1.96 ±0.16	41.61	n.d.
	SP	0	traces	n.d.	traces	0.01 ±0.00	0.13 ±0.00	n.d.	0.38 ±0.00	0.09 ±0.00	0.53 ±0.01	n.d.
		0.5	traces	n.d.	traces	0.02 ±0.00	0.28 ±0.00	0.01 ±0.00	0.85 ±0.00	0.10 ±0.01	1.16 ±0.01	n.d.
120 °C. 60 min	LP	0	0.17 ±0.01	n.d.	0.18 ±0.01	0.53 ±0.06	5.99 ±1.25	n.d.	21.41 ±1.87	1.88 ±0.15	28.28 ±3.20	362.88 ±12.41
	SP	0	traces	n.d.	traces	0.22 ±0.00	2.59 ±0.66	n.d.	8.57 ±0.16	0.88 ±0.22	11.39 ±0.72	n.d.
150 °C. 60 min	LP	0	0.16 ±0.02	n.d.	0.29	0.58 ±0.01	7.83 ±0.08	0.11 ±0.01	23.06 ±0.34	6.74 ±0.13	32.03 ±0.46	1667.09 ±4.64
		0.5	0.14 ±0.01	n.d.	0.25 ±0.02	0.54 ±0.01	8.64 ±0.06	n.d.	22.78 ±0.20	4.16 ±0.43	32.35 ±0.30	3083.09 ±9.82
	SP	0	traces	n.d.	traces	traces	0.03 ±0.00	n.d.	0.06 ±0.00	0.04 ±0.00	0.09 ±0.01	n.d.
		0.5	traces	n.d.	traces	traces	0.03 ±0.00	n.d.	0.08 ±0.00	0.05 ±0.00	0.12 ±0.01	n.d.

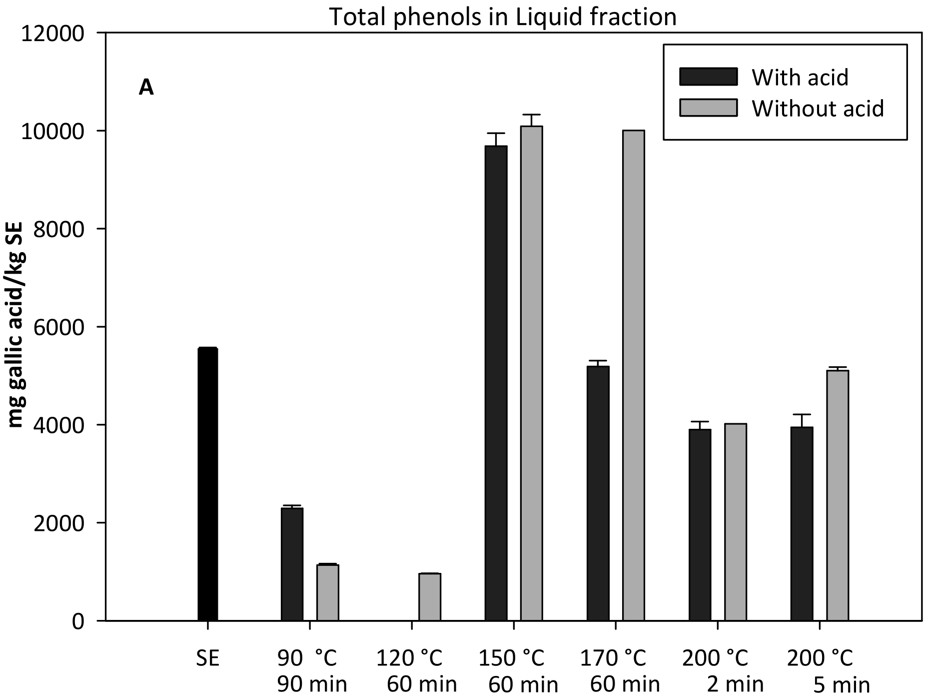
Table 2.1. Glycoside composition (mg/kg of fresh strawberry extrudate), total monosaccharides (Total MS) and total oligosaccharides (Total OS) and hydroxymethylfurfural (HMF) of the liquid phase (LP) and the solid phase extracted by alcoholic solution for all the hydrothermally treated samples and the untreated strawberry extrudate (SE) as a control. The analyzed sugars are Rhamnose (Rha), Fucose (Fuc), Arabinose (Ara), Xylose (Xyl), Mannose (Man), Galactose (Gal) and Glucose (Glu). n.d. non detected. Traces: values <0.00. *0.005 meaning value between 0.001 and 0.004. Standard deviations in brackets.

Treatment	Phases	% Acid	Rha	Fuc	Ara	Xyl	Man	Gal	Glu	Total OS	Total MS	HMF
170 °C. 60 min	LP	0	0.28 ±0.01	0.09 ±0.00	0.80 ±0.01	0.53 ±0.02	7.22 ±0.27	0.18 ±0.00	18.93 ±0.68	4.22 ±0.23	28.04 ±0.99	7651.81 ±4.56
		0.5	0.29 ±0.00	0.11 ±0.01	0.67 ±0.01	0.51 ±0.01	6.77 ±0.01	0.22 ±0.01	17.61 ±0.05	4.51 ±0.29	26.17 ±0.10	9370.97 ±46.22
	SP	0	0.01 ±0.00	traces	0.01 ±0.00	0.01 ±0.00	0.13 ±0.00	Traces	0.31 ±0.01	0.09 ±0.01	0.47 ±0.02	232.83 ±2.02
		0.5	traces	traces	0.01 ±0.00	0.01 ±0.00	0.068 ±0.00	traces	0.18 ±0.00	0.07 ±0.00	0.27 ±0.01	191.82 ±10.28
200 °C. 2 min	LP	0	0.09 ±0.00	0.03 ±0.00	0.12 ±0.00	0.16 ±0.00	1.88 ±0.00	0.03 ±0.00	5.79 ±0.06	1.92 ±0.00	8.10 ±0.07	351.93 ±37.71
		0.5	0.18 ±0.00	0.07 ±0.01	0.26 ±0.01	0.40 ±0.02	5.56 ±0.11	0.09 ±0.00	16.33 ±0.69	5.05 ±0.24	22.88 ±0.84	1045.51 ±3.58
	SP	0	traces	traces	traces	traces	0.04 ±0.00	traces	0.11 ±0.01	0.05 ±0.00	0.16 ±0.02	n.d.
		0.5	traces	n.d.	traces	traces	0.05 ±0.00	n.d.	0.14 ±0.00	0.04 ±0.00	0.20 ±0.01	n.d.
200 °C. 5 min	LP	0	0.21 ±0.01	0.09 ±0.00	0.37 ±0.01	0.384 ±0.00	4.42 ±0.06	0.12 ±0.00	12.94 ±0.18	3.55 ±0.28	18.54 ±0.26	3950.11 ±7.51
		0.5	0.16 ±0.00	0.07 ±0.00	0.28 ±0.00	0.259 ±0.00	3.06 ±0.02	0.09 ±0.00	8.59 ±0.18	3.28 ±0.24	12.51 ±0.20	3310.86 ±5.66
	SP	0	traces	traces	0.01 ±0.00	0.01 ±0.00	0.07 ±0.00	traces	0.18 ±0.00	0.07 ±0.00	0.26 ±0.01	10.07 ±1.18
		0.5	traces	traces	traces	traces	0.05 ±0.00	traces	0.13 ±0.00	0.03 ±0.00	0.18 ±0.00	2.35 ±0.65

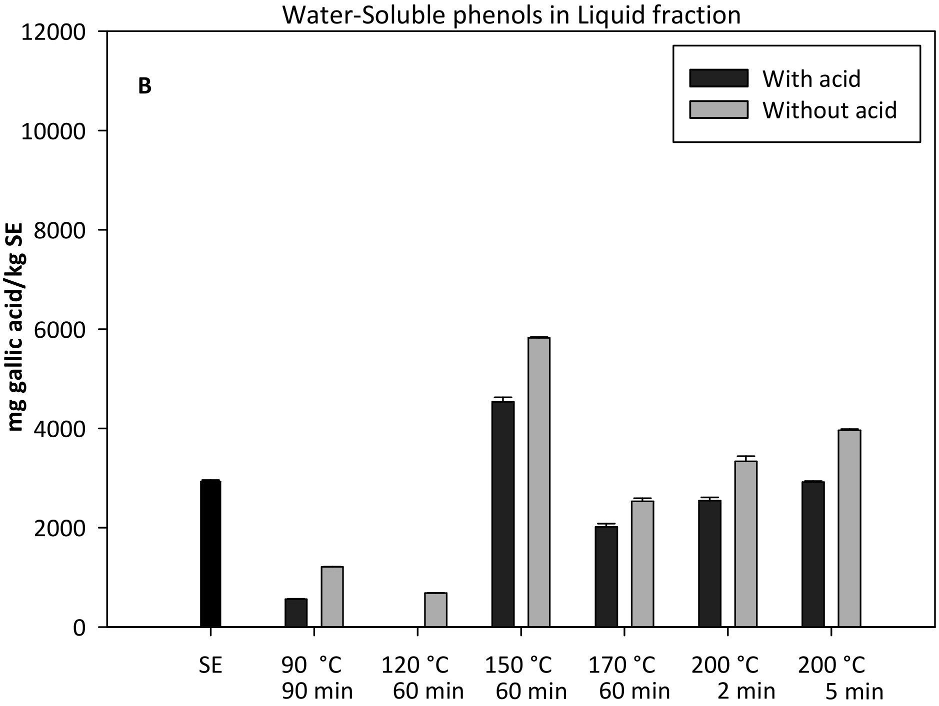
The glycoside composition (**Table 2.1**) shows that the solubilized individual sugars were extracted mainly in the liquid phase. The major sugar present in the liquid phase was glucose, followed by mannose, in all conditions tested. No significant amounts of sugars linked to the solid fraction were found except for the sample treated at 120 °C, in which around 40 % of the quantified sugars were retained in the solid fraction. Most probably this treatment enhanced the linked formation between the solubilized sugars from hemicellulose with the rest of the cell wall material. The maximum concentrations of mono- and oligosaccharides were obtained by treatment at 90 °C with acid addition, whereas the same treatment conditions without acid addition yielded 2-fold lower concentrations. In conditions without acid addition, the concentration of sugars increased at higher temperatures, reaching a maximum concentration of oligosaccharides at 150 °C, 21 % of which were monosaccharides. In contrast to the result obtained for treatment at 90 °C, the use of acid at 150 °C did not significantly increase the solubilization of sugars. In addition, the concentration of HMF in the liquid phase treated at 150 °C increased compared to the sample treated at 90 °C. Batch hydrothermal treatments between 90 °C and 170 °C obtained 26–41 g of monosaccharides/kg of fresh strawberry extrudate, whereas in a continuous hydrothermal treatment, it would be possible to obtain a liquid source with a much higher concentration of fermentable sugars, over 100 g/L, because the condensed water is lower.

2.3.2. Solubilization of phenols

The total phenols transferred in the liquid phase after hydrothermal treatment, representing the simple and complex phenols that are soluble in a mix of ethanol/water were quantified (**Figure 2.3 A**). The concentration of total phenols in the SE, i.e., 5.55 g/kg SE was lower than that present in the liquid fractions following treatment at 150 °C and 170 °C without acid addition, i.e., 10.0 mg/kg. The concentration of total phenols in the liquid fractions at 150 °C and 170 °C were much higher than for the other tested conditions (**Figure 2.3 A**), suggesting that phenols required not only a high temperature to be solubilized but also a longer reaction time, over one hour. The addition of acid at 150 °C led to the solubilization of 9.68 mg/kg, a value not significantly different than the maximum value determined without acid addition. The steam explosion treatment was not efficient at releasing a high concentration of phenols, most probably due to the short reaction time. Thus, the maximal recovery of phenols from strawberry extrudate was obtained with conditions in the industrial range (150 °C–170 °C), similar to those currently in use to treat other agro-wastes (Rubio-Senent et al. 2013).



Total phenols in Liquid fraction



Water-Soluble phenols in Liquid fraction

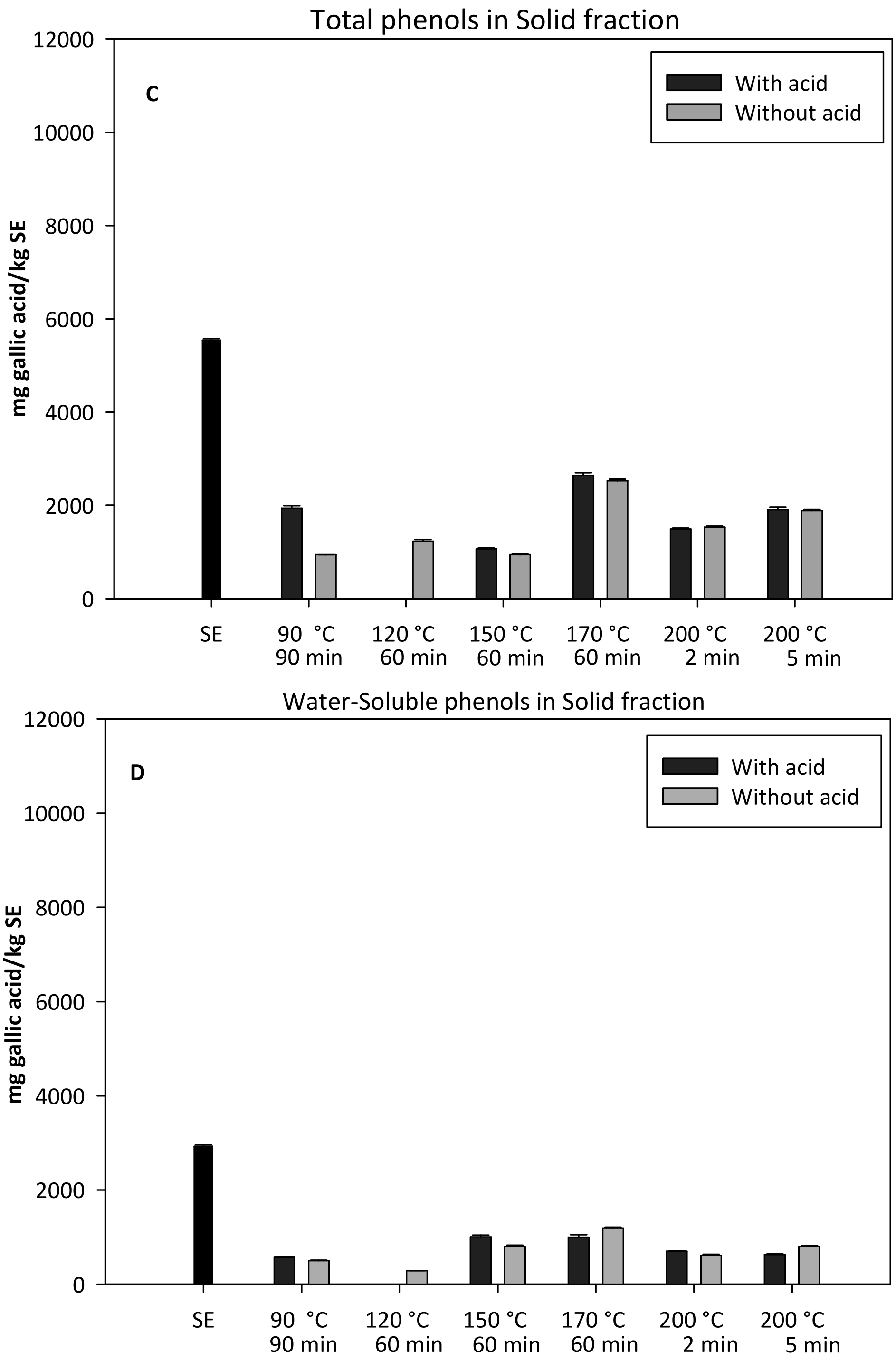


Figure 2.3. The quantity in milligrams of total phenols (expressed as gallic acid) in the liquid phase soluble in ethanol (A) or soluble in water (B) and in the solid phase extracted with ethanol (C) or water (D) that can be extracted from 1 kg of fresh strawberry extrudate using the hydrothermal treatment is shown. Untreated strawberry extrudate

(SE) was used as control. Means with the same letter were not significantly different, $p < 0.05$.

In comparison with the phenols soluble in ethanol/water, less than half of the total phenols in the liquid fraction (**Figure 2.3 A**) were soluble in water (**Figure 2.3 B**); therefore, these compounds must be complex phenols. The total phenol content (**Figure 2.3 C**) and the concentration of phenols soluble in water (**Figure 2.3 D**) in the solid fraction were much lower than those contained in the liquid fraction (**Figure 2.3 A and B**).

2.3.3. Antioxidant assays

The ability of each liquid fraction to scavenge the free radical DPPH was expressed as the concentration of extract (in mg per mL) necessary to decrease the initial concentration of $DPPH^{\bullet}$ by 50 % (IC50). Low IC50 values represent high antioxidant activity (Rodríguez et al. 2007). All the treatments at temperatures above 120 °C produced liquid fractions with significantly higher antioxidant activity than the SE (**Figure 2.4 A**), because the higher solubilization of phenols and the formation of other antioxidant compounds like HMF. No correlation was observed between the antioxidant activity of the samples and their phenol concentration, unlike the results reported for other agro-wastes after hydrothermal treatment (Rubio-Senent et al. 2012). However, there was a positive correlation with the antioxidant activity when the phenol concentration was considered together with the HMF concentration, which also has antioxidant activity (Mrabet et al. 2016). When also considering the HMF concentration, the highest antioxidant activity was found for the liquid fraction produced by treatment at 150 °C without acid addition for 60 min, almost 5 mg/mL, although this antioxidant activity was lower than those reported for extracts obtained with the same conditions from dates (0.3 mg/mL) or from olive oil waste (1.7 mg/L) (Rubio-Senent et al. 2013; Mrabet et al. 2016). In the cases of other agro-waste, the extracts were further purified; hence the antioxidant activity of the liquid fraction of strawberry extrudate could potentially be improved by purification.

Reducing power results are expressed as Trolox equivalents in mg/mL and high concentrations of Trolox equivalents indicate high reducing power activity. All liquid fractions showed higher reducing activity than the control, with the maximum activity detected for the liquid fraction obtained after treatment at 90 °C without acid addition (**Figure 2.4 B**). Reducing activity decreased with increasing temperature for no acid addition conditions. Addition of acid resulted in a lower reducing activity for all conditions tested compared to the non-acid addition conditions, with the exception of steam explosion treatment at 200 °C for 5 min. The reducing power values for hydrothermally-treated strawberry extrudate show the potential use of the phenol extracts that could be obtained from the liquid fraction. However, these values correspond to half the activity of the liquid fraction obtained from one of the most active phenol sources, olive oil waste. The phenol compounds present in the liquid fraction obtained from strawberry extrudate showed two of the most important mechanisms on which antioxidant activity is based: the ability to inhibit the formation of free radicals and the ability to scavenge any free radicals formed (Fraga et al. 2010).

2.3.4. Physicochemical characterization of the solid phase

The solid phases obtained after each hydrothermal treatment were analyzed in order to determine their potential use as biomass for further bioprocess applications. The use of the solid phase in industrial processes such as anaerobic digestion could be complementary not only for the final stabilization of strawberry waste, but also to obtain an energy source (methane) necessary to power the hydrothermal treatment reactor.

After hydrothermal treatments, the pH values of the solid fraction increased slightly due to the addition of water during direct heating, even without the addition of acid (**Table 2.2**). On average, 91 % of total solids were volatile solids. The low concentration of mineral solids was mainly due to the presence of salts and/or carbonates. The humidity increased after hydrothermal treatment because the capacity of the cell material to retain water also increased (Fernández-Bolaños et al. 2004). The highest humidity corresponded to the solid fractions obtained from the steam explosion reactions, in which the severity for lignocellulosic materials is higher than the other tested conditions (Fernández-Bolaños et al. 2004). The total chemical oxygen demand (COD) concentration of the solid phase reached up to 87 % for the treatments at 150 °C and 170 °C compared to the SE (**Table 2.2**). The high temperature and the long reaction time led to the solubilization of desirable compounds into the liquid fraction, with a high organic content remaining in the final solid residue. The addition of acetic acid did not improve the COD in any of the conditions tested (**Table 2.2**).

For all the treatments tested, the values of sCOD/COD ratio were close to zero because most of the organic matter was solubilized (**Table 2.2**). The values demonstrate the potential of the solid phase of hydrothermally-treated strawberry extrudate to be used as a source rich in organic matter for the application of anaerobic digestion or other bioprocesses for the complete valorization of this agro-waste after hydrothermal treatment at 160 °C for 60 min (Rubio-Senent et al. 2013).

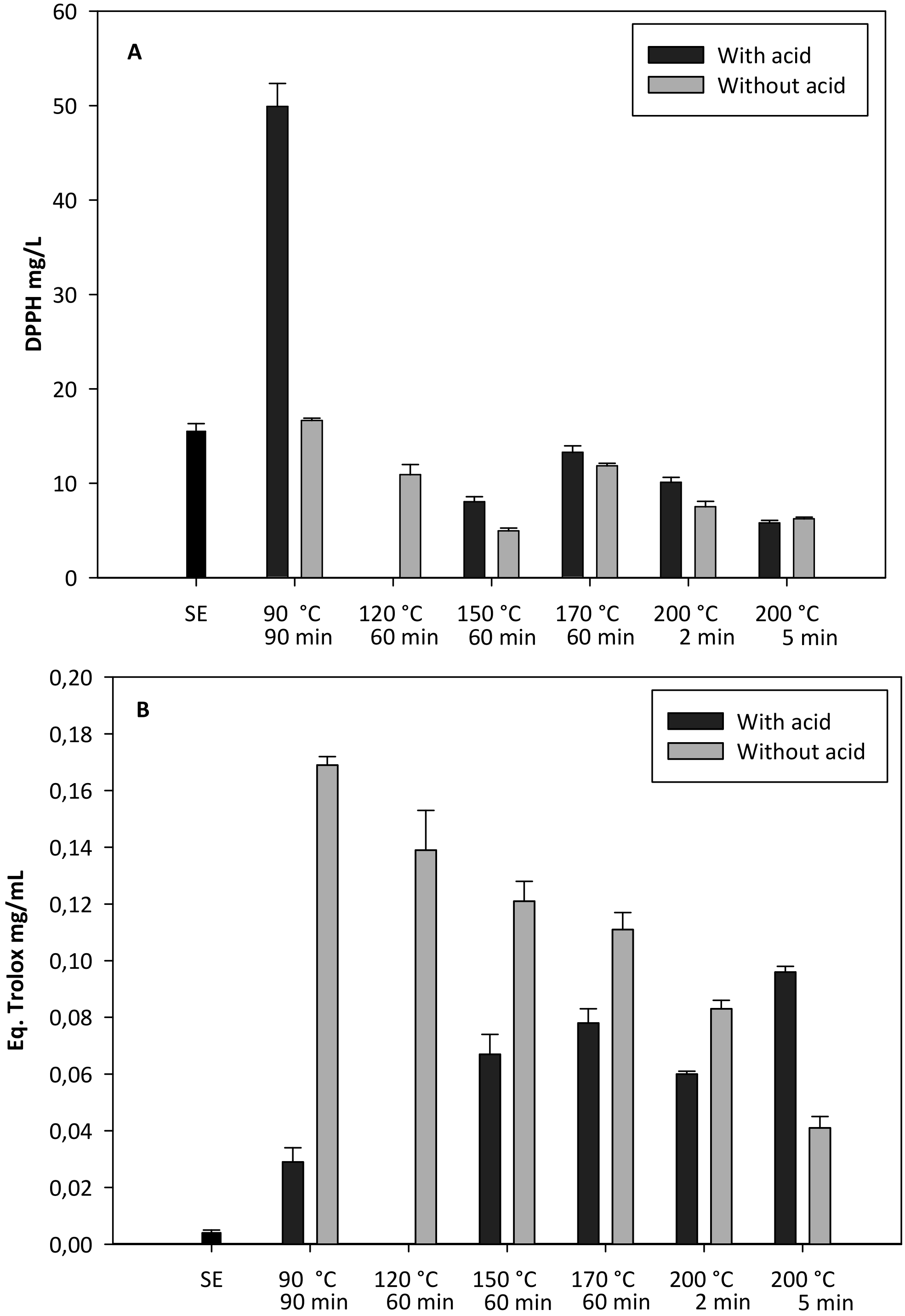


Figure 2.4. Antioxidant activities determined by DPPH (A) and reducing power (B) methods of each liquid fraction obtained after the hydrothermal treatments. Untreated strawberry extrudate was used as control (SE). Means with the same letter were not significantly different, $p < 0.05$.

Table 2.2. Physicochemical characterization of untreated strawberry extrudate (SE) and different solid fractions obtained after the hydrothermal treatments (Chemical Oxygen Demand (COD), soluble COD (sCOD), total solids (TS), mineral solids (MS), humidity (H) and total volatile solids (VS)). Standard deviations in brackets.

Treatment	% Acid	pH	TS (mg/Kg)	MS (mg/Kg)	VS (mg/Kg)	%H	COD (mg O_2/kg)	sCOD (mg O_2/L)	Ratio (sCOD/COD)
SE	-	3.3 ±0.1	156356 ±916	7482 ±87	148874 ±834	84.4 ±0.5	200953 ±6793	56460 ±2498	0.28
90 °C. 60 min	0	4.0 ±0.1	138847 ±6158	4850 ±161	133.997 ±6047	86.1 ±3.8	142898 ±4564	20768 ±616	0.15
	0.5	3.8 ±0.1	120644 ±1474	4313 ±101	116332 ±1568	87.9 ±1.1	126533 ±5403	22977 ±453	0.19
120 °C. 60 min	0	3.6 ±0.1	171287 ±1996	6078 ±129	165209 ±2109	82.9 ±1.0	196287 ±7137	27955 ±442	0.14
150 °C. 60 min	0	4.0 ±0.1	205296 ±2615	4235 ±234	201.062 ±2381	79.5 ±1.0	258084 ±2368	9510 ±476	0.04
	0.5	0.14 ±0.01	194085 ±4526	3855 ±66	190229 ±4464	80.6 ±1.9	261723 ±3245	12141 ±341	0.05
170 °C. 60 min	0	3.6 ±0.1	205787 ±3040	5278 ±80	200508 ±3116	79.4 ±1.2	307863 ±9058	24260 ±1058	0.08
	0.5	3.4 ±0.1	283920 ±12645	6898 ±476	277022 ±12170	71.6 ±3.2	376426 ±7871	27020 ±1011	0.07
200 °C. 2 min	0	4.5 ±0.1	91622 ±2151	1874 ±330	89748 ±1887	90.8 ±2.1	123355 ±2519	16097 ±802	0.13
	0.5	4.1 ±0.00	102035 ±2751	1736 ±172	100299 ±2579	89.8 ±2.4	90104 ±2120	7050 ±160	0.08
200 °C. 5 min	0	4.4 ±0.1	73039 ±2438	1879 ±11	71160 ±2427	92.7 ±3.1	83715 ±3233	32872 ±1620	0.39
	0.5	4.1 ±0.1	75585 ±3557	1573 ±130	74012 ±3648	92.4 ±4.4	108518 ±3283	7119 ±350	0.07

2.3.5. Mass balance

Based on the sugar and phenol solubilization as well as the antioxidant capacity of the liquid phase, the two most appropriate hydrothermal treatments for the valorization of strawberry extrudate were 150 °C and 170 °C for 60 min without acid addition. The phenol and sugar solubilization were roughly the same for both treatments, while the acid sugar solubilization was higher at 150 °C. The DPPH assay showed a high antioxidant activity for the liquid phase obtained at 150 °C, while the reducing power method did not show any difference between the two treatments. The concentration of undesirable compounds of sugar degradation like HMF was lower for the treatment at 150 °C. Thus, the use of treatment at 150 °C for 60 min led to better results than treatment at 170 °C, in addition to the consequent energy savings. **Figure 2.5** summarizes the mass balance of hydrothermal treatment at 150 °C for 60 min in a discontinuous system to valorize the strawberry extrudate. From 1 kg of extrudate, a liquid fraction that was rich in sugars (57 g) and phenols (10 g) was produced, plus 0.4 kg of final solid that could potentially be used for methane production by anaerobic digestion to produce the energy necessary to power the hydrothermal treatment reactor. About the HMF content 1.7 g/kg of SE, the dietary human intake of HMF has been estimated in a range of 2.1–23 mg/day (Rufián-Henares and de la Cueva 2008). Therefore, the maximum consumption should be 13.5 g/day of SE treated at 150 °C, 1 h, being this quantity high because the antioxidants are used in food in a very low concentration. Besides, the concentration of HMF could be diminished in the SE by economic system such as chromatographic columns commonly used in the food industry (Lama-Muñoz et al. 2012).

It is important to note that treatments at 150 °C in this study were carried out in a discontinuous reactor without a proper preheating, hence a high amount of water condensed during the reaction. In contrast, nowadays, most industrial hydrothermal treatments are carried out in more efficient continuous reactors (Fernández-Bolaños et al. 2010), such as those used for olive oil waste treatment, in which the water added by steam condensation only increases the initial humidity of the sample by 1 %. The volume of liquid generated during the reaction under industrial conditions is expected to be lower, and thus the concentration of sugars and phenols solubilized in the liquid phase of strawberry extrudate is expected to be even higher than the values reported in this study, as is the extract's antioxidant activity.

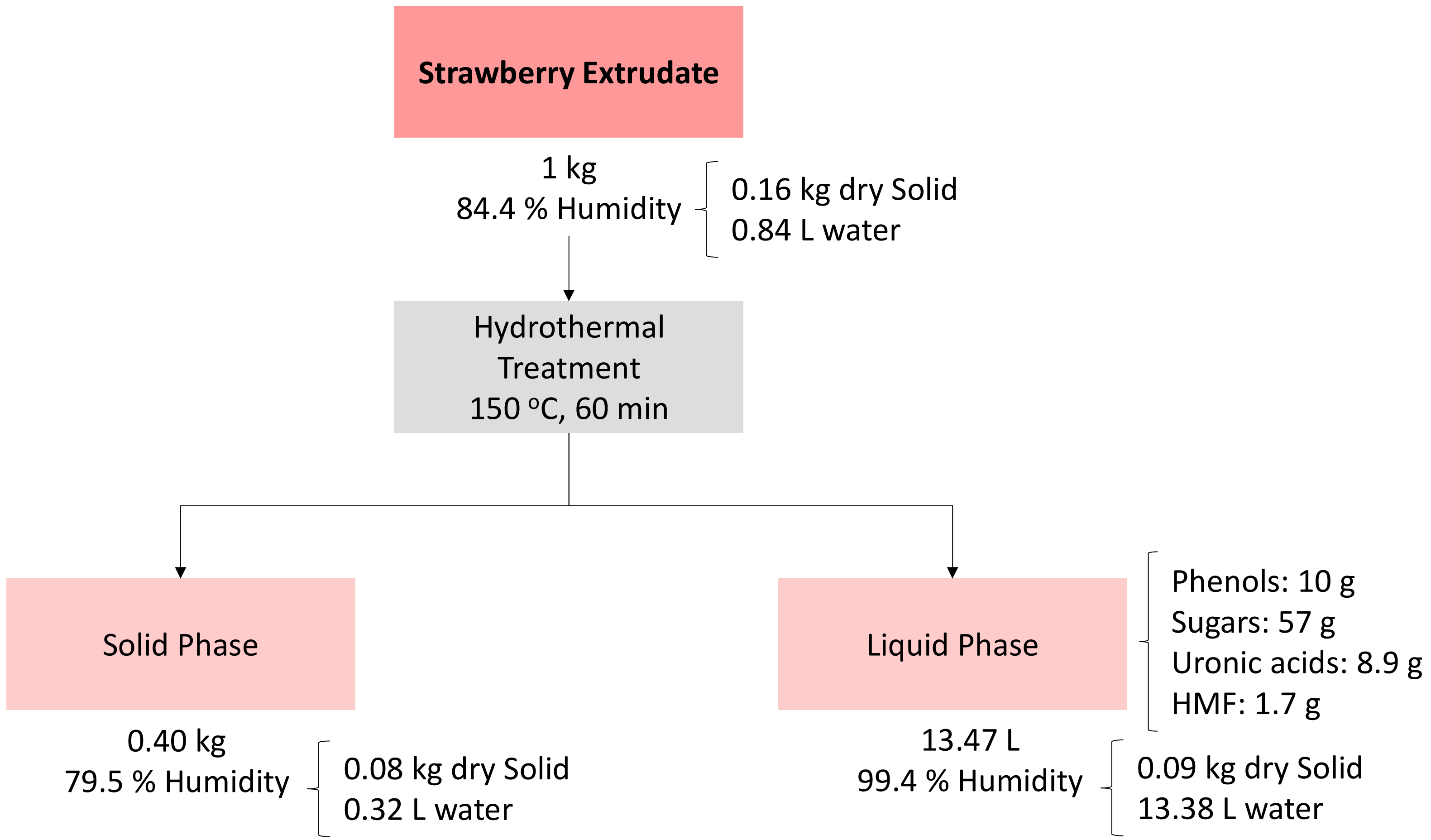


Figure. 2.5. Mass balance for the treatment at 150 °C for 60 min of the strawberry extrudate. HMF: hydroxymethylfurfural

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3

Extraction of phenol compounds and production of biomethane from strawberry and raspberry extrudates

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 phenol compounds was extracted from each extrudate through the adsorption-desorption processes, i.e., 876, 392, and 2,402 mg of gallic acid/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.

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3.1. 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 (Bobinaite et al. 2015; Nour et al. 2017). During the 2016 season, more than 12 million tons of berries were obtained in the world (FAO 2018). 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) (FAO 2018). 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 fibers part and the achenes. Currently, 21 and 5 % of strawberries and raspberries production, respectively, are destined to the manufacture of transformed products (Serrano et al. 2014; MAPAMA 2018). 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 phenol compounds (Giampieri et al. 2012; Basu et al. 2014; Bobinaite et al. 2015). The phenol anthocyanins and ellagitannins are the major antioxidant phytochemicals present in strawberries and raspberries (Rao and Snyder 2010; Giampieri et al. 2012). Other compounds present in these berries are uronic acids, which indicates the existence of acidic carbohydrates or pectins (Côté and Hahn 1994). Out of all compounds contained in the berries, phenol compounds are the most interesting due to their strong antioxidant capacity (Dias et al. 2017). Therefore, an interesting management option might be the recovery of these phenol bioactive compounds still present in the strawberry and raspberry extrudates.

Solubilization of the phenol compounds from the extrudates is necessary in order to recover them. Several methods have been recently proposed to extract phenol compounds from strawberry such as high hydrostatic pressure extraction, microwave hydro-diffusion and gravity, Pulsed Electric Field with solvent and hydrothermal treatments (Fermoso et al. 2018) (**Chapter 2**). Hydrothermal pre-treatment has been previously proposed for solubilization and extraction of phenols from other agro-waste such as olive mill solid waste (Serrano et al. 2017a), the bagasse of the wine (Soto Álvarez 2015) or strawberry extrudate (**Chapter 2**). It should be kept in mind that most phenol compounds present in strawberry and raspberry extrudates are thermosensitive (Struck et al. 2016), therefore, after the hydrothermal pre-treatment phenol 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 by-products such as furfural or 5-hydroxymethylfurfural (5-HMF), which can be inhibitory for anaerobic digestion processes at certain concentrations (Serrano et al. 2017a) (**Chapter 2**).

The phenol 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 phenol compounds with a further anaerobic digestion of the remaining biomass. The stabilized digestate produced after anaerobic digestion might be used as fertilizer component (Fermoso et al. 2018). 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 phenol 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 phenol compounds and subsequent anaerobic digestion process of the remaining biomass.

3.2. Materials and methods

3.2.1. 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 °C) before their use in order to prevent their self-fermentation and deterioration.

3.2.2. Hydrothermal pre-treatment

Hydrothermal pre-treatments were performed using a steam treatment batch reactor (100 L) and can reach temperatures up to 190 °C 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 °C in the reactor for 60 min. After the pre-treatments, samples were cooled to 25 °C 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 °C before characterization.

3.2.3. Extraction of phenol compounds

Phenol 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 %.

3.2.4. 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 phenol 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 °C), 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) (**Equation (1)**), which has been applied by other authors (Donoso-Bravo et al. 2010; Pinto-Ibieta et al. 2016; Serrano et al. 2017b) using the following expression:

$$B = B_{max} * \left(1 - exp\left[\frac{R_m(t - \Lambda)}{B_m}\right]\right) \qquad \textbf{Equation (1)}$$

where B (mL CH_4/g VS) is the cumulative specific methane production, B_{max} (mL CH_4/g VS) is the ultimate methane production, R_{max} is the maximum methane production rate (mL CH_4/ (g VS/d)), t (d) is the time and Λ (d) is the lag time. The second kinetics model used is the Logistic model (Sigmoidal parameter 4) (equation (2)), which has been applied by other authors (Donoso-Bravo et al. 2010; Li et al. 2012; Rincón et al. 2013) using the following expression:

$$B = B_0 + P/\left[1 + exp\left(-4 \cdot R_{max} \cdot (t - \Lambda)/(P + 2)\right)\right] \qquad \textbf{Equation (2)}$$

where B 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_{max} 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_{max} is the maximum methane production rate (mL CH_4/g SV d) and Λ (d) is the lag time. Additionally, R^2, error (%) and standard error of estimate (σ_{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_{max} (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_{max} values, because of the experiments were not carried out at the same time and different inoculums were used. R_{max} 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.

3.2.7. 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 (APHA 2012). 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 (Raposo et al. 2008), while soluble COD (sCOD) was determined by the closed digestion and the colorimetric standard method 5220D (APHA 2012).

3.2.7.1. Total phenols content

Content of total phenols was determined by Folin-Ciocalteu spectrophotometric method (Singleton and Rossi 1965) after an extraction with methanol/water solution (80:20) at 70 °C. Samples preparation included either centrifugation at 400 g during 5 min and subsequent filtration through 0,45 µm filter (García et al. 2016). Results were expressed as milligrams of gallic acid equivalents per kilogram of extrudate.

3.2.7.2. Total sugars and uronic acids

Antrone colorimetric method was used for determining total sugars (Mokrash, L. 1954) using a spectrophotometer (Biorad iMark Microplate Reader, USA). Samples preparation included either centrifugation at 400 g during 5 min, and subsequent filtration through 0,45 µm filter. Results were expressed as milligrams of glucose equivalents per kilogram of extrudate.

M-Hydroxybiphenyl Chromogen Method, as described by Blumenkrantz and Asboe-Hansen (Blumenkrantz and Asboe-Hansen 1974) was used for quantifying uronic acids. Results were expressed as grams of galacturonic acid equivalents per kilogram of extrudate.

3.2.7.3. Individual neutral sugars

Using a method described by Lama-Muñoz, Rodríguez-Gutierrez, Rubio-Senent, and Fernández Bolaños (Lama-Muñoz et al. 2012) 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).

3.2.8. 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}) (**Chapter 2**). 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.

3.2.9. 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 (Serrano et al. 2017a). Lower calorific power of the methane was equal to 35,793 J/L (Serrano et al. 2015). The efficiency in the energy obtained through a cogeneration biogas engine was considered as 33 % and 55 % for electricity and thermal energy, respectively (Cano et al. 2014). In order to reach the required operating temperature (35 °C) a specific heat of 4.18 kJ/kg·°C was considered to obtain the thermal energy requirement to increase the waste temperature from 20 to 35 °C, 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 (Ferrer et al. 2009).
- Hydrothermal pre-treatment. Enthalpy values of 104.9 (water 20 °C and 1 kg/cm^2) and 2,745.7 kJ/kg (steam 150 °C 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 % (Cano et al. 2014).
- 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 (Serrano et al. 2017a). The phenols 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 (Eurostat 2017). Methane: 0.04 €/kW (Fermoso et al. 2018).

3.3. Results and discussion

3.3.1. Characterization of the untreated extrudates

The physicochemical characterization of untreated strawberry and raspberry extrudates is showed in **Table 3.1**. 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 phenol 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 (Borja et al. 1996, 1997; Chen et al. 2008; Schroyen et al. 2018).

The three extrudates had a pH value around 3 (**Table 3.1**), 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 (2017). SE1 and SE2 had 85 % humidity, whereas the RE had 75 % humidity. This humidity difference may be due to the fact that raspberry has more fibers than strawberry, which are compounds with lower humidity (Bobinaite et al. 2015). 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 sCOD/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 3.1**). 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 (Schnürer and Jarvis 2010). SE1 contained a higher concentration of total sugars compared to SE2 and RE (**Table 3.1**). 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 3.1** 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 (Jönsson and Martín 2016). Uronic acids in SE2 were probably generated by oxidation of monosaccharides during the pasteurization process.

3.3.2. 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 phenol extract.

3.3.2.1. Effect of the hydrothermal pre-treatment on extrudates composition

Table 3.2 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. Humidity in SP from SE1 and SE2 was not modified with respect to untreated berry extrudates, while in the case of SP from RE the humidity 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 3.2**). 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.

Table 3.1. 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
Humidity (%)	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/g 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
sCOD (mg O_2/g VS)	400 ± 5	340 ± 10	100 ± 5
Ratio (SCOD/COD) (%)	33	24	10
Ratio C/N	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 3.2. Physicochemical characterization of untreated strawberry and raspberry extrudates and different fractions obtained after the hydrothermal pre-treatments and extraction of phenol compounds.

Phases	pH	TS (mg/kg Ext.)	MS (mg/kg Ext.)	VS (mg/kg Ext.)	Humidity (%)	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 3.3. 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 ± 1
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 ± 1
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 ± 1
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 ± 1
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 ± 1

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 (**Figure 3.1**). This was expected as most of sugars are soluble in water (**Chapter 2**). 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 phenol 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 (Hornedo-Ortega et al. 2017) 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 3.3**). Sugars contained in cellulose and hemicellulose were most likely produced and solubilized during the hydrothermal pre-treatment, as seen in other studies (Garrote et al. 1999; Hendriks and Zeeman 2009) (**Chapter 2**). As counterpart of the hydrothermal pre-treatment, part of the sugars is known to form Hydroxymethylfurfural (HMF) (**Table 3.3**). 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 (Fernández-Cegrí et al. 2012; Ghasimi et al. 2016). 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 (**Chapter 2**). 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 (Jönsson and Martín 2016). **Figure 3.2** 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.

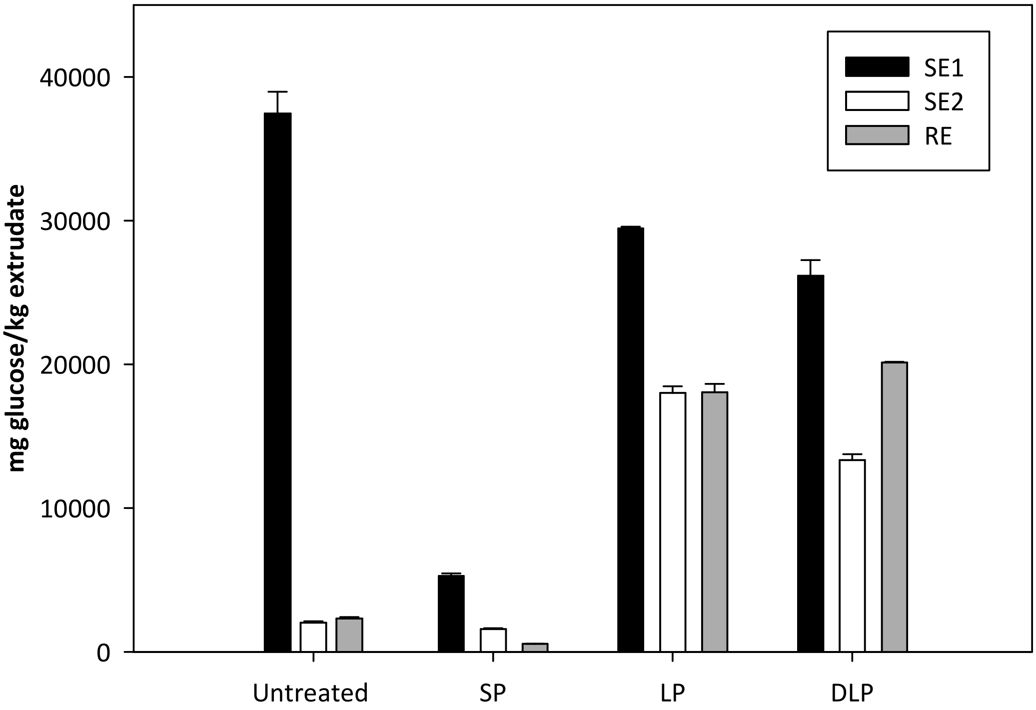

Figure 3.1. Total sugars expressed as milligrams of glucose per kilogram of extrudate for untreated strawberry and raspberry extrudates and different fractions obtained after the hydrothermal pre-treatments and extraction of phenol compounds.

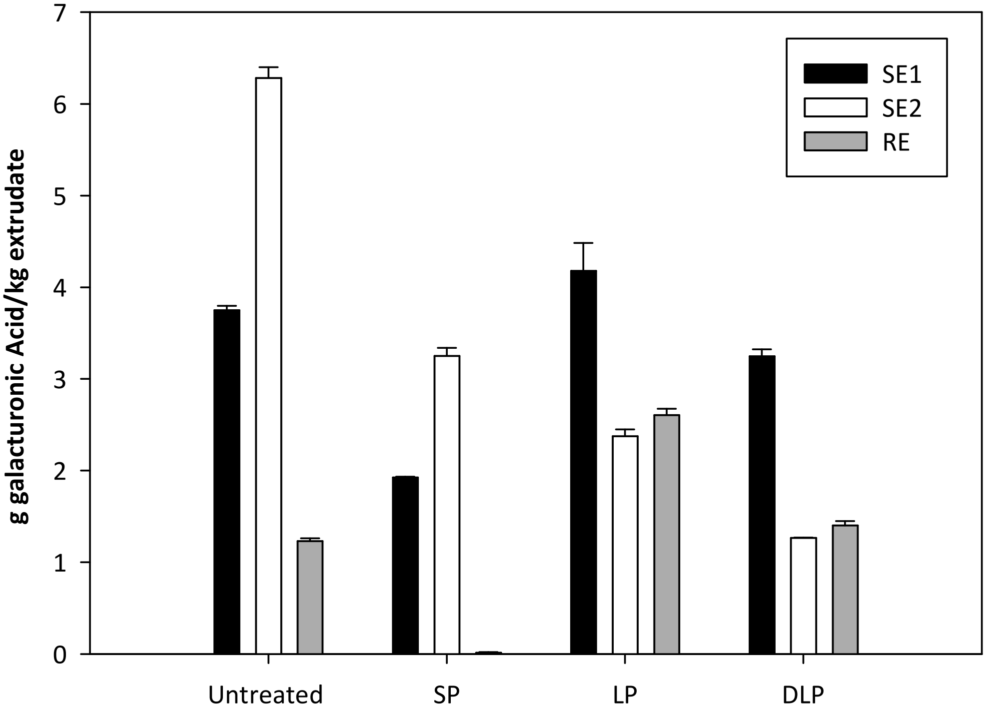

Figure 3.2. Uronic acids expressed as grams of galacturonic acid per kg of extrudate for untreated strawberry and raspberry extrudates and different fractions obtained after the hydrothermal pre-treatments and extraction of phenol compounds.

3.3.2.2. Effect of the hydrothermal pre-treatment on phenol 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 (**Figure 3.3**). 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 (Ariza et al. 2016). 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 (**Figure 3.4 A**). 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/kg extrudate in SE1, SE2 and RE, respectively. Therefore, the recovery efficiency 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 phenol compounds, antiradical activity increased in DLP, indicating a lower antioxidant capacity compared to LP (**Figure 3.4 A**). Similarly, reducing power in DLP decreased compared to LP (**Figure 3.4 B**). 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.

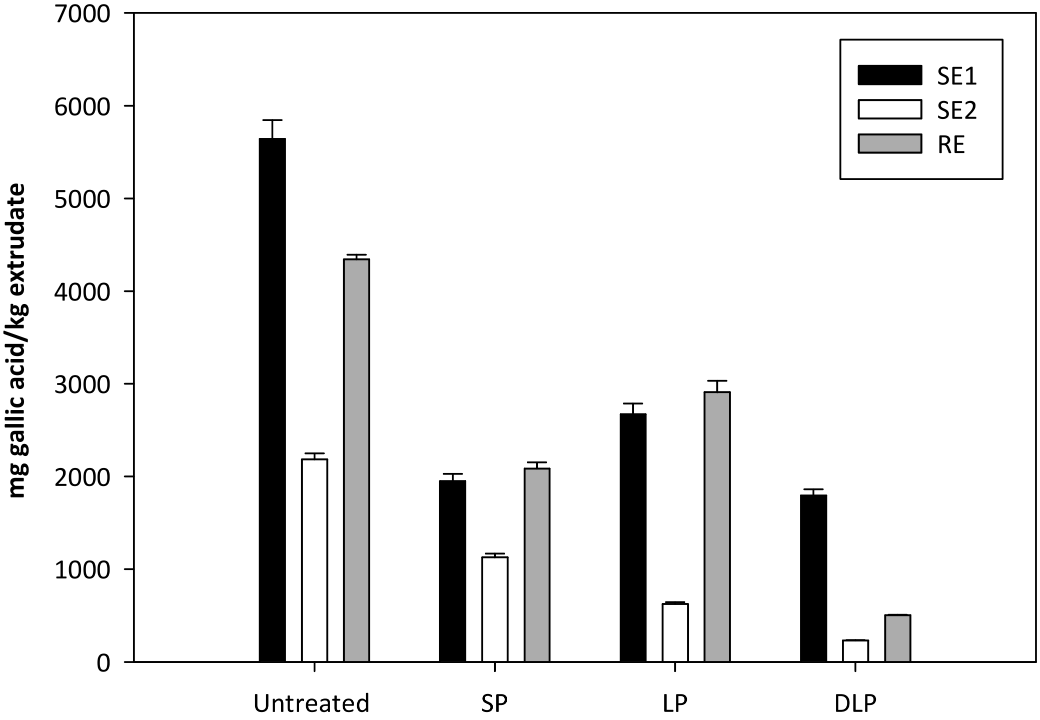

Figure 3.3. Total phenols expressed as milligrams of gallic acid per kg of extrudate for untreated strawberry and raspberry extrudates and different fractions obtained after the hydrothermal pre-treatments and extraction of phenol compounds.

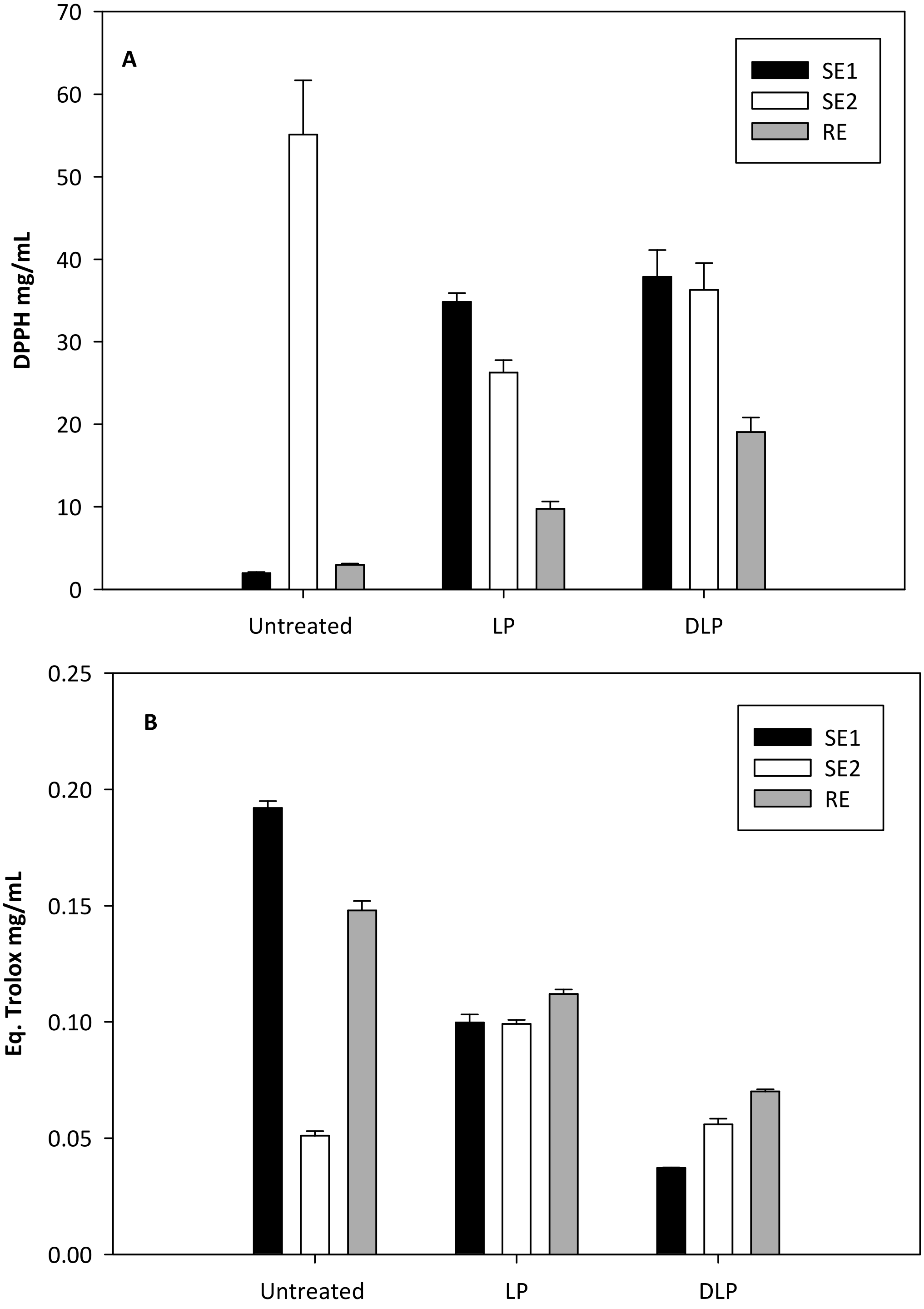

Figure 3.4. Antioxidant capacity determined by antiradical activity (DPPH) **(A)** and reducing power **(B)** methods of each LP obtained after hydrothermal pre-treatments and of each DLP obtained after extraction of phenol compounds.

3.3.3. Assessment of digestion stability, methane yield and methane production rate after hydrothermal pre-treatment and subsequent phenol 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 3.4** 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 (Wheatley 1990). 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 3.3**). These values were in all cases markedly lower than the reported inhibition concentration of HMF for anaerobic digestion process, i.e., 800 mg/L (Ghasimi et al. 2016).

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 (**Figure 3.5 A and B**), which entails an increase of 28.6 % when the substrate is hydrothermally pre-treated and the phenol 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 (**Figure 3.5 A and B**), which entails an increase of 19 % when the substrate is hydrothermally pre-treated, and the phenol 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 (**Figure 5 A and B**), which entails an increase of 11 % when the substrate is hydrothermally pre-treated, and the phenol compounds are extracted. **Table 3.5** shows the values of methane production rate, R_{max}, 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 3.5**). 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 3.5**). R_{max} for the untreated SE1 was 14.3 % higher than that obtained for the mixture of SP and DLP from SE1. Opposite to this, R_{max} for the mixture of SP and DLP from SE2 was 34.1 % higher than that obtained for untreated SE2 (**Table 3.5**), similar to the R_{max} 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 the microorganisms, since it was observed that the substrates that produced the greatest amount of methane were those of larger particles (SE1 and the mixture of SP and DLP from SE1, sieve 1.5 mm). Theoretically, the smaller the size of the particles, the greater the degradability of the matter by the microorganisms (Yadvika et al. 2004), since, logically, microorganisms can more easily degrade particles of smaller size and more hydrolyzed than particles with a larger size, which they must break down and previously degrade. The result obtained in this study was already described by other authors (De la Rubia et al. 2011), who pointed out that smallest particles contained a higher concentration of recalcitrant compounds (compounds resistant to biodegradation) than particles with a larger size.

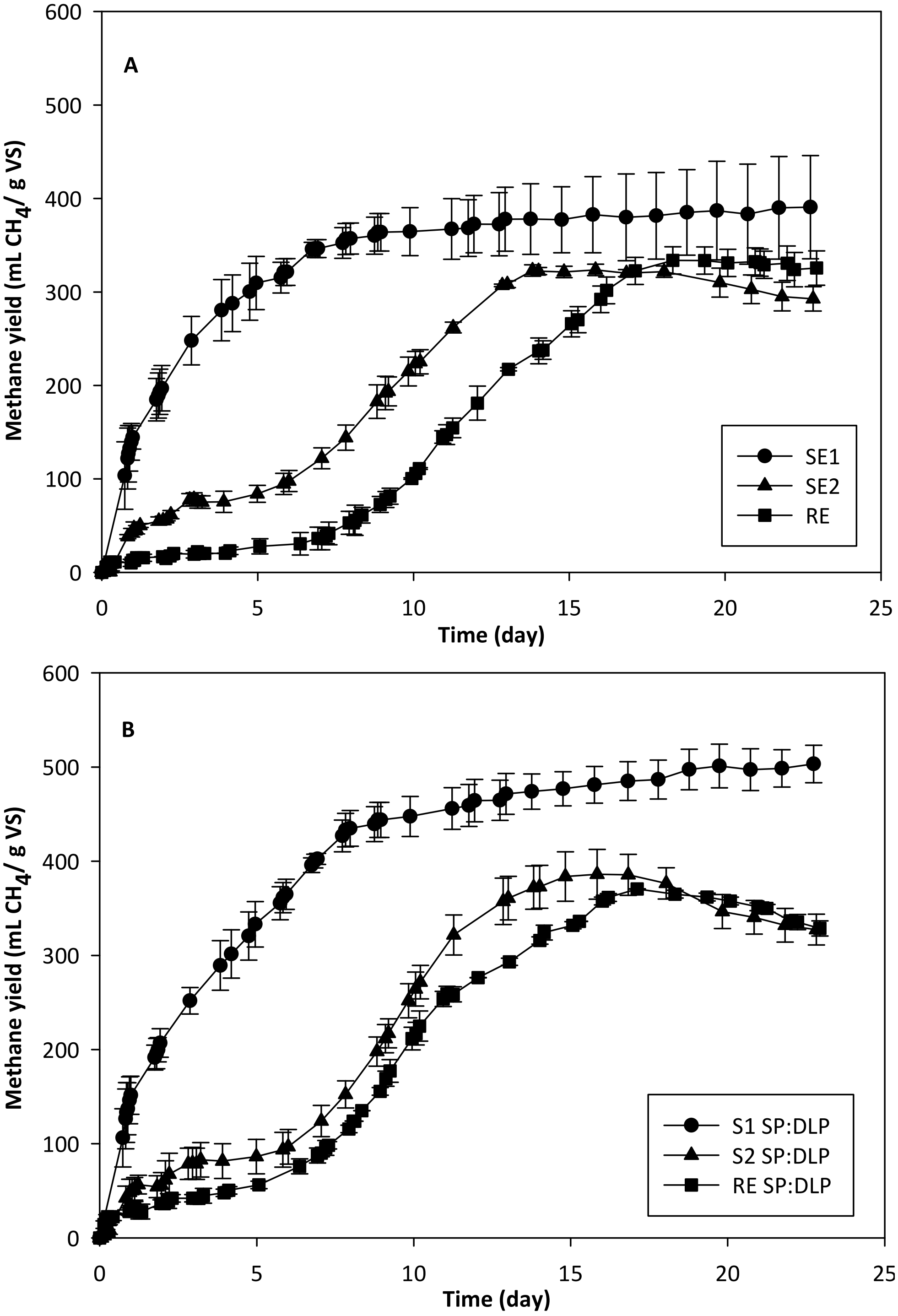

Figure 3.5. Methane production (mL CH_4/g VS) of untreated extrudates **(A)** and of pre-treated extrudates **(B).**

3.3.4. Economic assessment

The minimum sales price of phenol extract for a zero net benefit, expressed as €/g gallic acid equivalents, was calculated for each of the studied extrudates, corresponding to cases 2, 4 and 6 of **Table 3.6**. The most economically favorable case corresponded to the RE, from 0.203 €/g gallic acid equivalents, thus indicating that the proposed biorefinery approach would be economically feasible. This was 63.5 % lower than in the case of SE1, where the minimum phenol sales price for profitability was 0.556 €/g gallic acid equivalents. In the case of SE2, the minimum sales price of the phenols was calculated to be 1.23 €/g gallic acid equivalents, several times higher than the most favorable case of RE. This may be due to the lower initial amount of phenol compounds present in SE2, compared to the other two cases (**Table 3.1**).

Although the steam consumed in RE, 2.93 kg steam/kg extrudate, was much higher than in the cases of SE1 and SE2, 1.93 and 0.44 kg steam/kg extrudate, respectively, the extracted phenol compounds are also much higher in RE, 2.40 g gallic acid/kg extrudate versus 0.88 g gallic acid/kg extrudate in SE1 and 0.39 g gallic acid/kg extrudate in SE2. In addition, the methane yield of RE was higher than in SE1 and SE2 (**Table 3.6**). According to the above, the higher steam consumption of the RE case was largely compensated by a higher methane yield and a better phenol extraction.

The high methane production and phenol recovery of any of the three substrates, make them viable substrates for the proposed biorefinery approach. So, in the same industrial plant could be treated without the need to make changes in the operational process. Likewise, although pre-treated extrudates generate greater methane production than untreated extrudates, if the extraction would not be possible, either due 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 3.6**.

Table 3.4. 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
sCOD (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 3.5. 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_{max} (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_{max} (mL CH_4/g VS·d)	144 ± 5	126 ± 5	41 ± 1	55 ± 2	38 ± 2	41 ± 1
λ (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 3.6. 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

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200 mL
±5%
150
100
50
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100
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4

High added value compounds recovery with high temperature hydrothermal treatment and steam explosion, and subsequent biomethanization of residual strawberry extrudate

Abstract

This study consisted on the comparison of hydrothermal treatments at 170 °C (steam injection) and 220 °C (steam-explosion) to solubilize the organic matter contained in the residual strawberry extrudate, focusing on phenol compounds, susceptible to be extracted, and sugars. After the extraction step, the remaining strawberry extrudate phases were subjected to anaerobic digestion to generate biogas that would compensate the energy requirements of the suggested hydrothermal treatments and to stabilize the remaining waste. Hydrothermal treatment at 220 °C allowed the recovery of 2,053 mg of gallic acid per kg of residual strawberry extrudate. By contrast, after hydrothermal treatment at 170 °C, only 394 mg of gallic acid per kg of residual strawberry extrudate were recovered. Anaerobic digestion processes were applied to the de-phenolized liquid phase and the solid phase together, which generated similar methane productions, i.e., around 430 mL CH_4/g volatile solids, after both 170 °C and 220 °C hydrothermal treatments. Taking into account the latest observation, hydrothermal treatment at 220 °C is a preferable option for the valorization of SE due to the high solubilization of valuable phenol compounds that can be recover.

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4.1. Introduction

The strawberry sector has seen exponential growth in recent years, reaching 8.3 million tons in 2018 (FAO 2019). The strawberry sector includes not only a direct marketing of fresh fruit but also obtaining by-products derived from strawberries, such as yogurts, juices, or jams (Gagneten et al. 2019). These by-products of strawberries generate a waste called residual strawberry extrudate (SE) that is currently dumped in landfill. SE is formed by achenes, fibers, and part of the juice of strawberries that is retained and rejected during the extrusion of the fruits to obtain a strawberry concentrate. This waste as well as the strawberry contains a wide variety of interesting bioactive compounds, with high concentrations of nutrients and phytochemicals, which could be recovered (Aaby et al. 2007; Giampieri et al. 2012). The great variety of nutrients and phytochemicals that strawberry contains is of great interest in our diet because they are beneficial to avoid or prevent different cardiovascular, neurological, cancerous, and other diseases (Giampieri et al. 2012; Ariza et al. 2016).

The recovery of high added value compounds would allow considering the SE as a by-product to be valorized instead of a waste to be treated. Different methods have been proposed to extract high added value compounds from strawberry, such as high-hydrostatic-pressure extraction (Cao et al. 2011), microwave hydrodiffusion and gravity (Turk et al. 2017), pulsed electric field (Odriozola-Serrano et al. 2008, 2009), solvents (Kajdžanoska et al. 2011) and hydrothermal treatments (**Chapter 2**). Hydrothermal treatments can be an attractive alternative to extract high added value compounds from the SE because the control of the operation conditions (mainly temperature, pressure, and time) allow different solubilization grades to be obtained in the treated substrate. Hydrothermal treatments at medium temperatures (150–180 °C) solubilize most of the hemicellulose in the lignocellulosic biomasses but have a more limited effect over lignin and cellulose (Garrote et al. 1999; Hendriks and Zeeman 2009). Alternatively, hydrothermal extraction at high temperatures and pressures (0.69–4.83 MPa) followed by a rapid decompression, so-called steam-explosion treatments, enhance the solubilization of cellulose with respect to the medium-temperature hydrothermal treatments, whereas the lignin can be also partially affected (Hendriks and Zeeman 2009). Furthermore, under steam-explosion conditions, the rupture of the hemicellulose–lignin bonds makes the treated substrate more accessible to the action of enzymes in a further biological process and, hence, more biodegradable (Zheng et al. 2014). This alternative would be very interesting to make accessible the compounds in both the SE fibers and in the achenes, which are more resistant than the fibers.

Hydrothermal treatments allow the separation of the hydrothermally treated substrates into a solid and liquid phase, facilitating the recovery of valuable compounds. Most of the soluble valuable compounds are displaced to the generated liquid phases, facilitating their subsequent recovery. In addition, an adequate hydrothermal treatment could increase the concentration of valuable compounds to be extracted from the liquid phase, such as phenols or sugars, from the breakdown of the lignocellulosic fibers (Rubio Senent 2011). The recovery of phenol compounds of the SE would be of great relevance, not only from the environmental point of view but also due to their application in different sectors of the alimentary and pharmaceutical industries (Rubio-Senent et al. 2013) (**Chapter 3**). Previously, in **Chapter 2** studied the effect of different hydrothermal treatments, i.e., at 90, 120, 150, and 170 °C during 60 min, and steam explosion at 200 °C (2 and 5 min), with and without acid addition, over strawberry extrudate. These authors observed a close positive relation in the hydrothermal treatments between the increment of the treatment temperature and the concentration of sugars and phenol compounds. However, it is necessary to evaluate the effect of the hydrothermal temperature over the stability of the phenol compounds, since some of the bioactive compounds in the strawberry have been described as thermosensitive (Terefe et al. 2013; Serrano et al. 2020). The recovery of the phenol compounds from the treated SE allows the partial detoxification of the substrate, but a large amount of sugar-rich organic matter remains. This de-phenolized SE must be correctly managed to avoid potential environmental risks associated with the uncontrolled putrefaction of the organic matter, such as greenhouse gas emissions, pollution of aquatic ecosystem due to lixiviates, etc. (Siles et al. 2013).

Anaerobic digestion is a valorization method that has been widely proposed for the stabilization of biodegradable substrates, which might be an interesting option to recover energy from SE and to stabilize the organic matter (Ward et al. 2008). The extraction of the phenol compounds solubilized during the hydrothermal treatments would be beneficial for the subsequent anaerobic digestion process due to the inhibitory effect of these compounds over the anaerobic microorganisms, especially the methanogens (Serrano et al. 2017; Millati et al. 2020). Previous studies have demonstrated that a hydrothermal treatment at 150 °C allowed the extraction of 392 mg of gallic acid/kg SE in relation to the phenols and has been able to increase 19.1 % of the methane potential compared to the untreated strawberry extrudate, showing that the biorefinery process proposed is a good option for handling this substrate (**Chapter 3**). Nevertheless, it must be borne in mind that the temperature increases can release soluble sugar-derived by-products such as furfural, 5-hydroxymethylfurfural (5-HMF), etc., which can be inhibitory for anaerobic digestion processes at certain concentrations (Serrano et al. 2017) (**Chapter 2**).

The aim of this research was to compare the effect of hydrothermal treatments at 170 °C for 60 min and steam explosion at 220 °C for 5 min on the SE characteristics. After the hydrothermal treatments and subsequent extraction of phenol compounds, anaerobic digestion was carried out by means of BMP (biochemical methane potential) tests to study the effects of these hydrothermal pre-treatments on the biomethanization of untreated and hydrothermally pre-treated strawberry extrudates.

4.2. Materials and methods

4.2.1. Residual strawberry extrudate

The SE was provided by Hudisa S.A, located in Huelva, Spain (37.281813, −7.239095, Huelva, Spain). Once collected, the SE was immediately stored at −20 °C to avoid the uncontrolled fermentation of the substrate.

4.2.2. Hydrothermal treatments systems and separation of liquid and solid phases

High-temperature hydrothermal treatments were carried out at temperatures of 170 and 220 °C. The first hydrothermal treatment was carried out at 170 °C and 5 kg/cm^2 pressure for 60 min by means of a steam-treatment reactor by direct steam injection. The second treatment was carried out at 220 °C and 32 kg/cm^2 pressure for 5 min, followed by a rapid decompression, in a pilot-scale steam-explosion reactor. Subsequently, the treated SEs were centrifuged to separate the liquid phase (LP) and solid phase (SP).

High-temperature hydrothermal treatments were carried out using a steam-treatment reactor with 100 L of capacity, which can reach temperatures up to 190 °C and a maximum pressure of 1.2 MPa. Heating of the strawberry extrudate was performed by direct steam injection. Wherein, 12.59 kg of SE was introduced in the reactor at 170 °C and 5 kg/cm^2 pressure for 60 min. After the treatment period, the sample was cooled to 50 °C and then centrifuged at 4700 g/1450 rpm (Comteifa, S. L., Barcelona, Spain) to separate the liquid phase (LP) and solid phase (SP). Steam-explosion treatments were performed in a pilot-scale reactor (Nusim, S.A., Madrid, Spain). The reactor was equipped with a stainless-steel deposit with 2 L of capacity. The steam-explosion reactor was loaded with 400 g of SE, which was heated at a temperature of 220 °C and 32 kg/cm^2 pressure for 5 min. An electronic computing device controlled the time and the temperature in a pre-programmed manner. After each treatment, the centrifuge (4226 Pacisa) was used to separate the liquid phase (LP) and solid phase (SP).

4.2.3. Extraction of phenol compounds

The extraction of the phenol compounds from the LP was carried out by a chromatographic method using adsorbent resin Amberlite XAD-16. The phase obtained after extraction has been called the de-phenolized liquid phase (DLP). The compounds adsorbed on the resin were subsequently extracted with 200 mL EtOH 80 % (v/v) and 40 mL EtOH 96 %.

The extraction of the phenol compounds was carried out with a column, 4.5 cm in diameter and 140 cm in height, filled with 100 mL of adsorbent resin Amberlite XAD-16. This Amberlite was dissolved in water corresponding to 12 cm in height in the column for the hydrothermal treatment of 170 °C, while in the hydrothermal treatment of 220 °C, a column 2 cm in diameter and 50 cm in height was used, filled with 25 mL of adsorbent resin Amberlite XAD-16 dissolved in water corresponding to 8 cm in height in the column. Amberlite XAD-16 is an adsorbent resin that retains a large percentage of phenols. These adsorption resins are highly crosslinked polymers with a large surface and numerous pores. In recent years, many studies have shown the efficacy of this type of resin in the design of new adsorption–desorption processes retaining a large number of bioactive compounds such as phenols (Soto et al. 2011). Extractions were carried out from 2 L of liquid phase after hydrothermal treatment at 170 °C and from 0.5 L of liquid phase after hydrothermal treatment at 220 °C. The phase obtained after extraction has been called the de-phenolized liquid phase (DLP). The compounds adsorbed on the resin were extracted with 200 mL EtOH 80 % (v/v) and 40 mL EtOH 96 % according to Fernández-Bolaños et al. (2011).

4.2.4. Anaerobic digestion experimental procedure

The anaerobic digestibility of untreated residual strawberry extrudate and the different phases after hydrothermal treatments and sequence extraction of phenols were evaluated by biochemical methane potential (BMP) tests. The BMP tests were carried out up to the total exhaust of the gas production (24 days period), which was monitored daily throughout the process.

The anaerobic digestibility of untreated residual strawberry extrudate and the different phases after hydrothermal treatments and sequence extraction of phenols were evaluated by biochemical methane potential (BMP) tests. BMP tests were carried out in 250 mL Erlenmeyer flasks using a working volume of 240 mL. In all cases, the BMP reactors were loaded with an inoculum/substrate ratio of 2:1, based on VS. All these assays were carried out in triplicate. 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 bath of thermostatic water under mesophilic conditions (35 °C), and they were continuously stirred by magnetic bars to favor the transfer of matter between inoculum and substrate. The produced biogas was passed through a 2 N NaOH solution to capture CO_2 and the remaining gas was assumed to be methane. The volume of methane was measured by liquid displacement. The BMP tests were carried out in the time interval required to exhaust gas production and VS removal (24 days period).

Methane production was monitored daily throughout the process. A sludge from the anaerobic treatment of wastewater from the beer industry of "HEINEKEN SPAIN, S. A.," (Seville, Spain) was used as an inoculum source. The main characteristics of the anaerobic inoculum were as follows: pH = 7.4 ± 0.1; alkalinity = 2500 ± 20 mg $CaCO_3$/L; TS = 72,000 ± 200 mg/kg; MS = 14,000 ± 350 mg/kg; VS = 58,000 ± 400 mg/kg.

4.2.5. Kinetic study

The kinetic parameters for each experiment were determined numerically from the experimental data obtained by non-linear regression with the SigmaPlot Software (version 11.0) (Systat Software Inc., San Jose, California, USA). The kinetic model used was the logistic model (sigmoidal 4 parameters), which was previously described by Donoso-Bravo et al. (2010).

The kinetic parameters and mathematical adjustment for each experiment were determined numerically from the experimental data obtained by non-linear regression, using the Software SigmaPlot (version 11.0). The kinetic model used was the logistic model (sigmoidal 4 parameters) (**Equation (1)**):

$$B = B_0 + P/\left[1 + exp\left(-4 \cdot R_{max} \cdot (t - \lambda)/(P + 2)\right)\right] \quad \textbf{Equation (1)}$$

where B is the cumulative methane production during the second stage (mL CH_4/g VS), B_0 is the cumulative methane production at the beginning of the second stage (mL CH_4/g VS), and should approximately coincide with the value of B_{max} obtained at the end of the first stage, P is the maximum methane production obtained in the second stage (mL CH_4/g VS), R_{max} is the maximum rate of methane production (mL CH_4/g VS·d), t (d) is the time and λ (d) is the delay time. In addition, R^2, error (%), and standard error of estimate (S.E.E.) were determined to evaluate the fit and accuracy of the results. The error was defined as the difference in percentage between the final experimental cumulative methane production and the theoretical value predicted by the model.

4.2.6. Chemical analyses

To measure the water-soluble compounds of the SE and of the solid phase (SP) after the hydrothermal treatments, an extraction was carried out by adding 160 g of distilled water to 20 g of sample and keeping it stirred for 24 h. After this time, the liquid was centrifuged and microfiltered with 0.45 µm nylon microfilters. This method allows to quantify the soluble compounds in the solid sample of this work and is also widely used for composting analysis (Ahmad et al. 2018). To extract the soluble compounds from the liquid phase (LP) and de-phenolized liquid phase (DLP), it was centrifuged again to remove solid-phase residues in suspension and microfiltered with 0.45 µm nylon microfilters. The following parameters were determined in the SE and/or in the effluents of the reactors: total chemical oxygen demand (COD), soluble COD (sCOD), total solids

(TS), mineral solids (MS), total volatile solids (VS), and pH. All determinations were carried out in accordance to standard methods (APHA 2017). The description of the analytical methods for the determination of total sugars, acid sugars, total phenols, and hydroxymethylfurfural (HMF) can be found in **Chapter 3**. The determination of the soluble compounds from the SP was carried out after a water extraction according to Thompson et al. (2002).

4.3. Results and discussion

4.3.1. Effect of hydrothermal treatments on the substrate characteristics

Table 4.1 summarizes the physicochemical characterization of the different phases obtained after carrying out the hydrothermal treatments, as well as the untreated SE. Regardless of the hydrothermal treatment, VS accounted more than 90 % of the TS in all the samples. The solubilization of organic matter through the hydrothermal treatments was evaluated by comparing the sCOD of the sum of the LP and SP (expressed as mg O_2/kg SE) to the sCOD in the untreated SE. According to the results, hydrothermal treatments increased the sCOD in comparison with untreated SE up to 23 % and 103 %, at 170 and 220 °C, respectively (**Table 4.1**). Most of these soluble compounds were displaced to the LP after the hydrothermal treatments, which retained around 75 % and 85 % of the sCOD, at 170 and 220 °C, respectively (**Table 4.1**). The increase in the solubilization of organic matter (sCOD) and the decrease in VS indicates that the treatment at 220 °C is more severe than the treatment at 170 °C. The conditions of the hydrothermal treatment had a high influence on the distribution of the total phenols between the SP and the LP. On one hand, after the hydrothermal treatment at 170 °C, total phenols were distributed at 57 % in the SP and 43 % in the LP (**Table 4.1**). It is worth noting that the sum of total phenols in LP and SP at 170 °C resulted in a loss of 8 % of total phenols with respect to untreated SE. On the other hand, at 220 °C, total phenols were distributed at 37 % in the SP and 63 % in the LP. On the contrary to treatment at 170 °C, total phenols increased around 180 % with respect to untreated SE after the hydrothermal treatment at 220 °C (**Table 4.1**). The decrease in the total phenol content at 170 °C could be explained by the thermosensitive character of some bioactive compounds in the strawberry, which are usually degraded at high temperatures (Terefe et al. 2013; Serrano et al. 2020). At 220 °C, although some thermosensitive compounds would be also degraded, the steam-explosion pre-treatment is able to alter the cellulose and, even, partially the lignin (Singh et al. 2015), releasing phenol compounds that were not available after less severe hydrothermal treatments (Song et al. 2018; Ahmad et al. 2018).

The increase in the concentration of total sugars, i.e., considering the sum of LP and SP, was almost five times higher after the treatment at 170 °C and around six times higher after the treatment at 220 °C, with respect to untreated SE (**Table 4.1**). Therefore, hydrothermal treatment at 220 °C allowed the solubilization of 37 % more total sugars than at 170 °C (**Table 4.1**). On the contrary, the acid sugars decreased after the hydrothermal treatments in comparison to the untreated SE, especially at 220 °C (**Table 4.1**). Other authors also reported a decrease in the concentration of acid sugars due to the application of high-temperature treatments (Jönsson and Martín 2016)(**Chapter 2**). As it was described for the phenol compounds, the increase in the concentration of total sugars is explained by the degradation of the lignocellulosic fibers of the SE during the hydrothermal treatments, especially at 220 °C (Ahmad et al. 2018; Song et al. 2019).

HMF poses well-known inhibitory properties for microorganisms (Ghasimi et al. 2016), which could limit the subsequent implementation of bioprocesses, such as anaerobic digestion (Hendriks and Zeeman 2009; Serrano et al. 2019). HMF was not detected in the untreated SE. After the hydrothermal treatments, the highest concentration of HMF was found at 170 °C, reaching a concentration of up to 2993 ± 29 mg/kg SE in LP. On the contrary, hydrothermal treatment at 220 °C only resulted in a concentration of 155 ± 2 mg/kg SE in LP despite the higher operational temperature compared to 170 °C. The formation of HMF is closely related with the application of high temperatures, which involves the conversion of carbohydrates to furans such as the HMF (Singh et al. 2015) (**Chapter 2**). The higher concentration of HMF at 170 °C with respect to 220 °C can be explained by the longer treatment time used in the first treatment (170 °C) compared to that used in the steam-explosion treatment (220 °C), i.e., 60 and 5 min, respectively, since the formation of HMF has been reported to be time dependent (Kowalski et al. 2013).

Table 4.1. Physicochemical characterization of untreated SE and different treated phases (mean values ± standard deviations; n.d.: non-detected).

	SE	**170 ^{0}C, 60 min, 5 kg/cm^2**		**220 ^{0}C, 5 min, 32 kg/cm^2**	
		SP	**LP**	**SP**	**LP**
pH	3.7 ± 0.1	4.3 ± 0.1	3.8 ± 0.1	3.6 ± 0.1	3.8 ± 0.1
TS (mg/kg SE)	144,681 ± 3986	94,280 ± 871	32,044 ± 1024	58,092 ± 1268	47,120 ± 1515
VS (mg/kg SE)	139,336 ± 4423	90,801 ±3405	29,430 ± 606	56,162 ± 2106	43,296 ± 891
COD (mgO$_2$/kg SE)	200,366 ± 6312	116,027 ± 1918	40,516 ± 373	89,775 ± 3220	63,910 ± 915
sCOD (mgO$_2$/kg SE)	47,237 ± 317	14,423 ± 463	43,927 ± 352	14,279 ± 316	82,027 ± 1194
Total phenols (mg gallic acid/kg SE)	2185 ± 64	1141 ± 30	858 ± 0	2200 ± 3	3895 ± 165
Total sugars (mg glucose/kg SE)	2023 ± 99	875 ± 23	12322 ± 222	1644 ± 34	13769 ± 178
Acids sugars (mg galacturonic acid/kg SE)	6.28 ± 0.12	2.11 ± 0.06	2.93 ± 0.06	0.29 ± 0.00	0.38 ± 0.01
HMF (mg/kg SE)	n.d.	73 ± 1	2993 ± 29	5 ± 1	155 ± 2

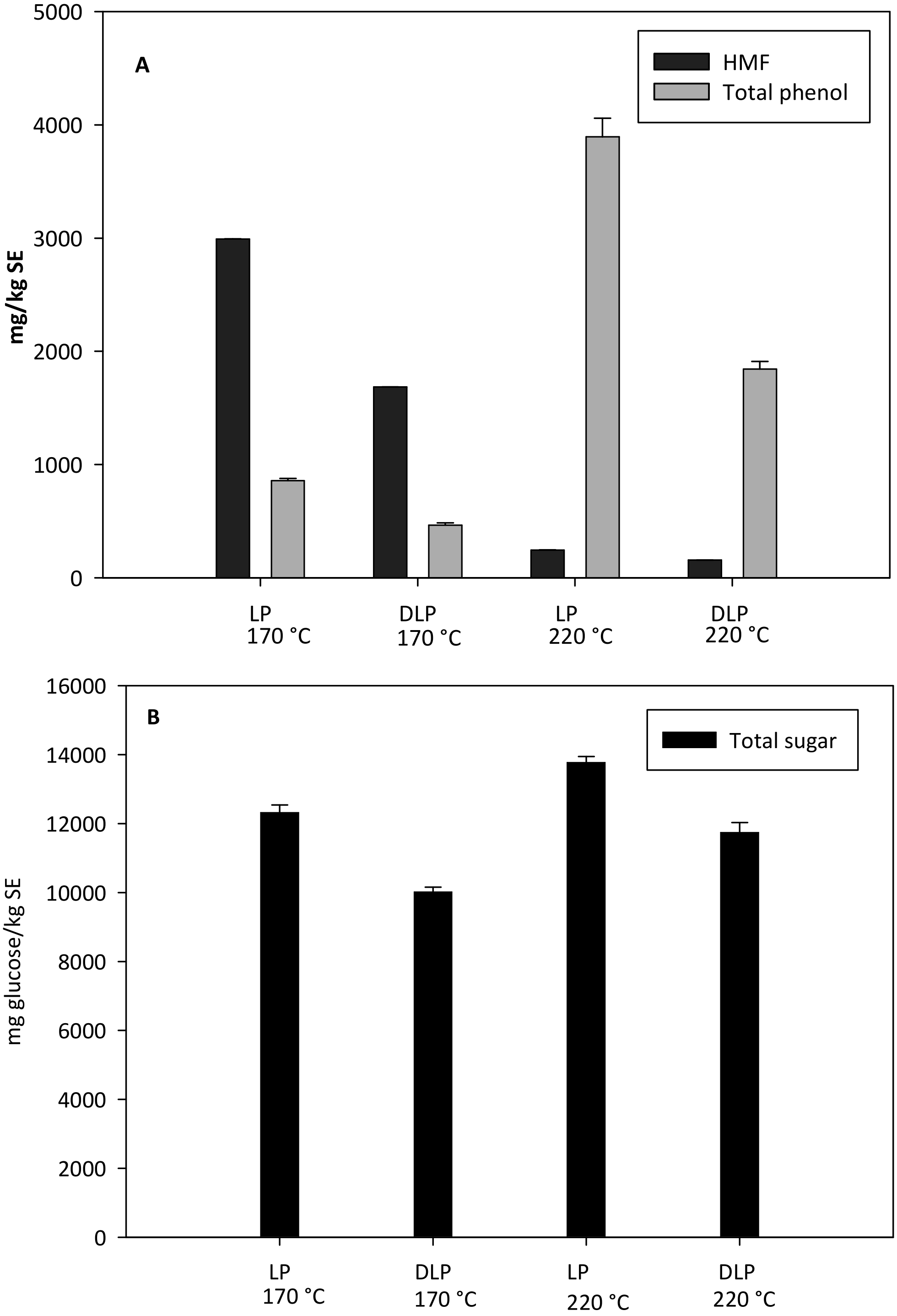

Figure 4.1. (A) Total phenols, HMF and (B) total sugars from LP and DLP after extraction of phenol compounds with their standard deviations

4.3.2. Effect of the extraction of phenol compounds in the liquid phase

The efficiency of the Amberlite XAD-16 resin used for the extraction of phenol compounds, i.e., total phenols and HMF, from LP is shown in **Figure 4.1A**. The extraction of total phenols presented an efficiency of up to 45 % and 53 % from the LP obtained at 170 and 220 °C, respectively (**Figure 4.1A**). These percentages were similar to those obtained with Amberlite XAD-16 resin for the extraction of phenol compounds from other substrates such as mulberry and agricultural waste (Tilay et al. 2008; Arfan et al. 2012). Amberlite XAD-16 resin was also able to remove up to 43 % and 37 % of HMF from the LP obtained at 170 and 220 °C, respectively (**Figure 4.1A**). Amberlite XAD-16 resin normally is used to extract hydrophilic and polar compounds, for this, it can be seen as having a good efficiency in the extraction of phenol and HMF compounds (Ahmad et al. 2015). However, the Amberlite XAD-16 resin did not affect to most of the total sugars in both LPs, retaining only around 18 % and 15 % of the total sugars at 170 and 220 °C, respectively (**Figure 4.1B**).

4.3.3. Anaerobic digestibility study after the application of hydrothermal treatments at high temperatures and subsequent extraction of phenol compounds

4.3.3.2. Methane potential and kinetic study of the anaerobic process after treatment at 170 °C

A BMP test was carried out to evaluate the biomethane production from SE, LP, SP, and DLP as well as the mixtures SP + LP and SP + DLP, after the hydrothermal treatment at 170 °C. **Table 4.2** shows the results of the methane production, as well as the characterization of the effluents of the BMPs at the end of the digestion time. As can be seen, pH was very similar for all the conditions with values between 7.7 and 7.8 (**Table 4.2**), and, thereby, within the recommended range for an adequate methanogenic activity, i.e., 7.3–7.8 (Wheatley 1990). The optimal pH values at the end of the experimental time, despite of the acidic character of the substrates (**Table 4.1**), indicated a proper buffering of the anaerobic digestion systems (BMPs), which presented alkalinity concentration values in a range of 5000–7000 mg $CaCO_3$/L.

Table 4.2 also shows that the amount of organic matter (VS and sCOD) after the anaerobic digestion processes of the different substrates was in all cases less than 10 g VS/kg and 1.5 g O_2/L, respectively. It also shows that the concentration of total phenols determined after the anaerobic digestion processes of the different substrates was in all cases less than 180 mg gallic acid/L, this concentration was much lower than those found as inhibitory in anaerobic digestion processes (Borja et al. 1996, 1997). Finally, **Table 2** also shows the biodegradability values, which were calculated from methane production. LP and DLP reached high degradation values, close to 100 %, while the SP had the lowest biodegradability (67 %). Co-digesting these phases, high biodegradability values of 81 % for SP + LP and 90 % for SP + DLP were achieved. It can be observed that after the extraction of phenol compounds a 9 % improvement in biodegradability was attained.

Figure 4.2 A and B shows the graph of cumulative methane production (mL CH_4/g VS) versus digestion time (days) for untreated SE and for LP, SP, and DLP after 170 °C hydrothermal treatment as well as for the mixtures SP + LP and SP + DLP. **Figure 4.2 A and B** shows that, at the beginning of the test, all samples had a small increase in methane production during the first 2 days. Although a slight difference was detected, since LP had a higher production in these initial two days compared to DLP, which could indicate that the extracted phenol compounds were easily biodegradable (**Figure 4.2 A**). The heterogeneity of the SE, where some components such as the fibers present a slow degradation, whereas some soluble compounds are easily biomethanized, could be responsible for the stepped curve in **Figure 4.2 A** (Ware and Power 2017). Next, from day 2 to day 7, approximately, a lag phase (latency period or adaptation) was observed in the curves of all substrates. From day 7 onward, continuous exponential growth was observed until, approximately, day 15, when production began to be constant for all samples. LP and DLP were the substrates that produced the highest methane productions, while the SP was the fraction that generated the lowest methane production. In **Figure 4.2 A**, a slight difference in the cumulative methane production between LP and DLP can be seen. The slightly higher cumulative methane production in DLP could be a consequence of the extraction process, mainly by the reduction of HMF with respect to the high concentration determined in LP (Hendriks and Zeeman 2009; Ghasimi et al. 2016). The combination of the SP + LP and SP + DLP gave methane productions similar to that achieved for untreated SE (**Figure 4.2 B**), which indicates that produced HMF did not significantly affect the overall methane production.

Table 4.2. Analytical characterisation of effluents from the anaerobic digestion process at the end of the BMP tests (after treatment at 170 °C) with their standard deviations.

	SE	SP	LP	DLP	SP+LP	SP+DLP
pH	7.7 ± 0.1	7.8 ± 0.1	7.8 ± 0.1	7.8 ± 0.1	7.7 ± 0.1	7.8 ± 0.1
Alkalinity (mg $CaCO_3$/L)	5768 ± 278	5902 ± 47	6174 ± 145	6590 ± 145	6049 ± 51	6028 ± 46
TS (mg/kg)	15,391 ± 442	15,570 ± 156	15,272 ± 118	15,057 ± 327	15,104 ± 265	15,610 ± 655
VS (mg/kg)	9918 ± 369	9961 ± 235	9250 ± 173	9226 ± 226	9837 ± 367	9981 ± 403
sCOD (mg O_2 /L)	1181 ± 48	1184 ± 56	1448 ± 59	1464 ± 17	1224 ± 79	1196 ± 65
Total phenols (mg gallic acid/L)	158 ± 8	163 ± 1	172 ± 6	171 ± 9	159 ± 14	157 ± 4
Experimental production (mL CH_4 /g VS)	416± 8	329 ± 7	497 ± 6	580 ± 7	403 ± 105	434 ± 32
Biodegradability (based on VS) (%)	74	67	94	125	81	90

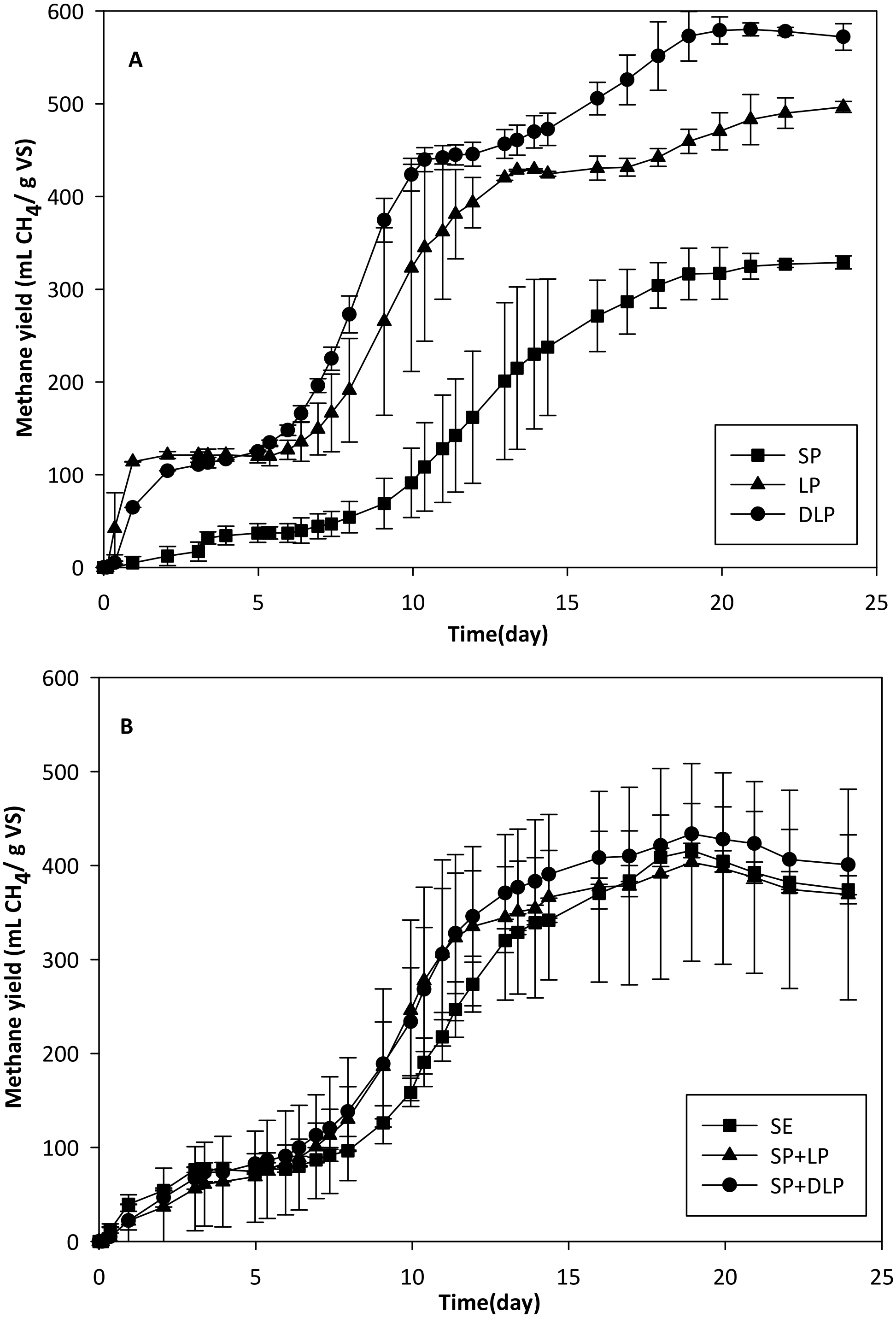

Figure 4.2. (**A**) Variation of cumulative methane production (mL CH_4/g VS) (VS, total volatile solids) with time for LP (liquid phase), SP (solid phase), and DLP (de-phenolized liquid phase) after 170 °C hydrothermal treatment with their standard deviations; (**B**): Variation of cumulative methane production (mL CH_4/g VS) with time for the untreated

SE (residual strawberry extrudate), SP + LP and SP + DLP mixtures after 170 °C hydrothermal treatment with their standard deviations.

Table 4.3 shows the values of the parameters obtained from the logistic model (sigmoidal 4 parameters) for the different substrates and mixtures studied after the hydrothermal treatment at 170 °C. The high values of R^2 obtained, as well as the low values of errors and standard error of estimates (S.E.E.) indicated that the experimental data fit correctly to the proposed model. The maximum methane production rate, designated by R_{max}, of the SP + LP mixture was 8.3 % higher than the maximum production rate of untreated SE (**Table 4.3**). The maximum methane production was reached for the mixture of SP + DLP, and the value of R_{max} for this mixture was 5.5 % higher than that for untreated SE (**Table 4.3**). Therefore, it can be said that applying the 170 °C treatment and performing the anaerobic digestion process after the hydrothermal treatment and subsequent extraction of phenols, no significant change both in the maximum methane production and maximum methane production rate were observed, and these values were similar both in the SE and in the mixture SP + DLP. **Table 4.3** also shows the lag time data (ʎ) for all the substrates assayed. As can be seen, and similar to what occurred for both the maximum methane production and maximum methane rate, the lag values were very similar for SE and for the mixture SP + DLP, showing the lowest value for DLP (8.3 days).

Table 4.3. Values of the parameters obtained from the Logistic model (Sigmoidal 4 parameters) for the different substrates and mixtures studied (hydrothermal treatment at 170 °C).

Phases	P (mL CH_4/g VS)	R_{max} (mL CH_4/g VS·d)	λ (d)	B_0 (mL CH_4/g VS)	R^2	Error (%)	S.E.E.
SE	345 ± 6	50.6 ± 4.4	11.45 ± 0.09	66 ± 4	0.9964	1.2	8.88
SP	305 ± 4	35.6 ± 1.5	12.37 ± 0.07	22 ± 3	0.9987	0.4	4.34
LP	351 ± 10	59.8 ± 9.3	9.5 ± 0.1	107 ± 8	0.9899	6.3	11.61
DLP	540 ± 54	52.4 ± 15.1	8.3 ± 0.5	22 ± 47	0.9755	3.0	10.9
SP + LP	341 ± 7	55.3 ± 5.6	9.5 ± 0.1	51 ± 6	0.9952	2.6	10.16
SP + DLP	364 ± 5	54.8 ± 4.1	10.02 ± 0.08	63 ± 4	0.9977	1.5	7.45

4.3.3.2. Methane potential and kinetic study of the anaerobic digestion process after hydrotreatment at 220 °C

BMP tests were carried out to evaluate the energy recovery from untreated SE, and for LP, SP, and DLP after 220 °C hydrothermal treatment, as well as for the mixtures SP + LP and SP + DLP. **Table 4.4** shows the values of the analytical parameters of the effluents obtained after the BMP tests for discussion of methane production, stability, and biodegradability. The stability of the anaerobic process was evaluated through pH and alkalinity values. The pH values, 7.6–7.8, remained within the recommended range for adequate methanogenic processes (from 7.3 to 7.8) (Wheatley 1990) (**Table 4.4**). The alkalinity values after the BMP tests varied between 5000 and 6000 mg $CaCO_3$/L, values high enough to buffer possible pH variations, as can be seen in the final pH values of the biomethanization processes. **Table 4.4** shows that the amount of organic matter (VS and sCOD) after the anaerobic digestion processes of the different substrates was in all cases less than 8.5 g VS/kg and 2 g O_2/L, respectively. It also shows that the concentration of soluble phenols found after the anaerobic digestion processes of the different substrates was in all cases less than 201 mg gallic acid/L and these concentrations were much lower than those studied as inhibitory in anaerobic digestion processes (Borja et al. 1996, 1997). Finally, **Table 4.4** also shows that the biodegradability (calculated from methane production) of the LP and DLP reached values around 100 %, while, on the contrary, the SP had the lowest biodegradability (45 %). The co-digestion of these phases achieved biodegradability values of 84 % for SP + LP and 78 % for SP + DLP, these biodegradability values were very similar to that achieved for untreated SE.

Figure 4.3 A and B shows the variation of cumulative methane production (mL CH_4/g VS) with digestion time (days) for untreated SE and for the LP, SP, and DLP after 220 °C hydrothermal treatment, as well as for the mixtures SP + LP and SP + DLP. **Figure 4.3** shows that at the beginning of the digestion time (2 days), except for the SP, a small increase in methane production was observed. Next, from day 2 to day 7, approximately, a lag phase (latency or adaptation period) was observed in the curves of all the substrates, except for the SP (**Figure 4.3 A**), for which the lag phase starts at the beginning of the assay. From day 13 for the SP and day 7 for the other phases, a continuous exponential growth was observed until reaching day 15, where the production begins to be constant for all the samples, except for SP where it began on day 18. LP and DLP were the substrates that produced more methane and SP the one with the lowest methane production (**Figure 4.3 A**). LP and DLP had similar cumulative methane production curves, despite the higher concentration of phenol compounds in LP with respect to DLP. The same trend was also reported in the methane production of LP and DLP obtained after similar hydrothermal treatment studies on olive-mill solid waste and raspberry extrudate (Serrano et al. 2017) (**Chapter 3**). The combinations of the SP + LP and SP + DLP gave methane productions similar to that of untreated SE, as occurred in the anaerobic digestion of these mixtures from hydrothermal treatment at 170 °C.

Table 4.5 shows the values of the parameters obtained from the logistic model (sigmoidal 4 parameters) for the different substrates and mixtures studied after hydrothermal treatment at 220 °C (steam explosion). As can be observed, the high values of R^2 obtained, as well as the low values of errors and standard errors of estimate (S.E.E.) indicated that the experimental data fit correctly to the proposed model. The maximum methane production rate, R_{max}, of the mixture SP + DLP was 43.9 % higher than the R_{max} value of the untreated SE (**Table 4.5**). With respect to the maximum methane production, the mixture SP + DLP produced 19.34 % less methane than that obtained for untreated SE (**Table 4.5**). Absence of the extracted phenol compounds improved production rate, however, the degradability of such compounds is also missed in the final methane production as seen in the slightly lower methane production value of SP + DLP compared to SE and the mixture SP + LP (**Table 4.5**). **Table 4.5** also shows the lag time data (ʎ) for each one of the substrates tested, this value was very similar for the SE and for the mixture SP + DLP and SP + LP.

Table 4.4. Analytical characterisation of effluents from the anaerobic digestion processes at the end of the BMP tests (after treatment at 220 °C) with their standard deviations.

	SE	**SP**	**LP**	**DLP**	**SP+LP**	**SP+DLP**
pH	7.7 ± 0.1	7.8 ± 0.1	7.7 ± 0.1	7.7 ± 0.1	7.7 ± 0.1	7.6 ± 0.1
Alkalinity (mg $CaCO_3$/L)	5617 ± 18	5328 ± 185	5482 ± 141	5333 ± 142	5328 ± 204	5264 ± 59
TS (mg/Kg)	12,987 ± 180	13,412 ± 667	12,533 ± 237	12,702 ± 211	13,082 ± 354	13,401 ± 290
MS (mg/Kg)	5478 ± 246	5062 ± 440	4988 ± 142	5343 ± 412	5331 ± 279	5276 ± 351
VS (mg/Kg)	7685 ± 256	8425 ± 180	7370 ± 307	7171 ± 276	7848 ± 345	7975 ± 572
sCOD (mg O_2 /L)	767 ± 33	1973 ± 89	1438 ± 39	1020 ± 62	1144 ± 63	937 ± 10
Total phenols (mg gallic acid/L)	134 ± 1	159 ± 2	201 ± 7	158 ± 9	176 ± 3	165 ± 5
Theoretical production (mL CH_4/g VS)	559	661	564	481	590	554
Experimental production (mL CH_4 /g VS)	468± 4	299 ± 12	562 ± 13	512 ± 30	493 ± 30	434 ± 27
Biodegradability (based on VS) (%)	84	45	100	106	84	78

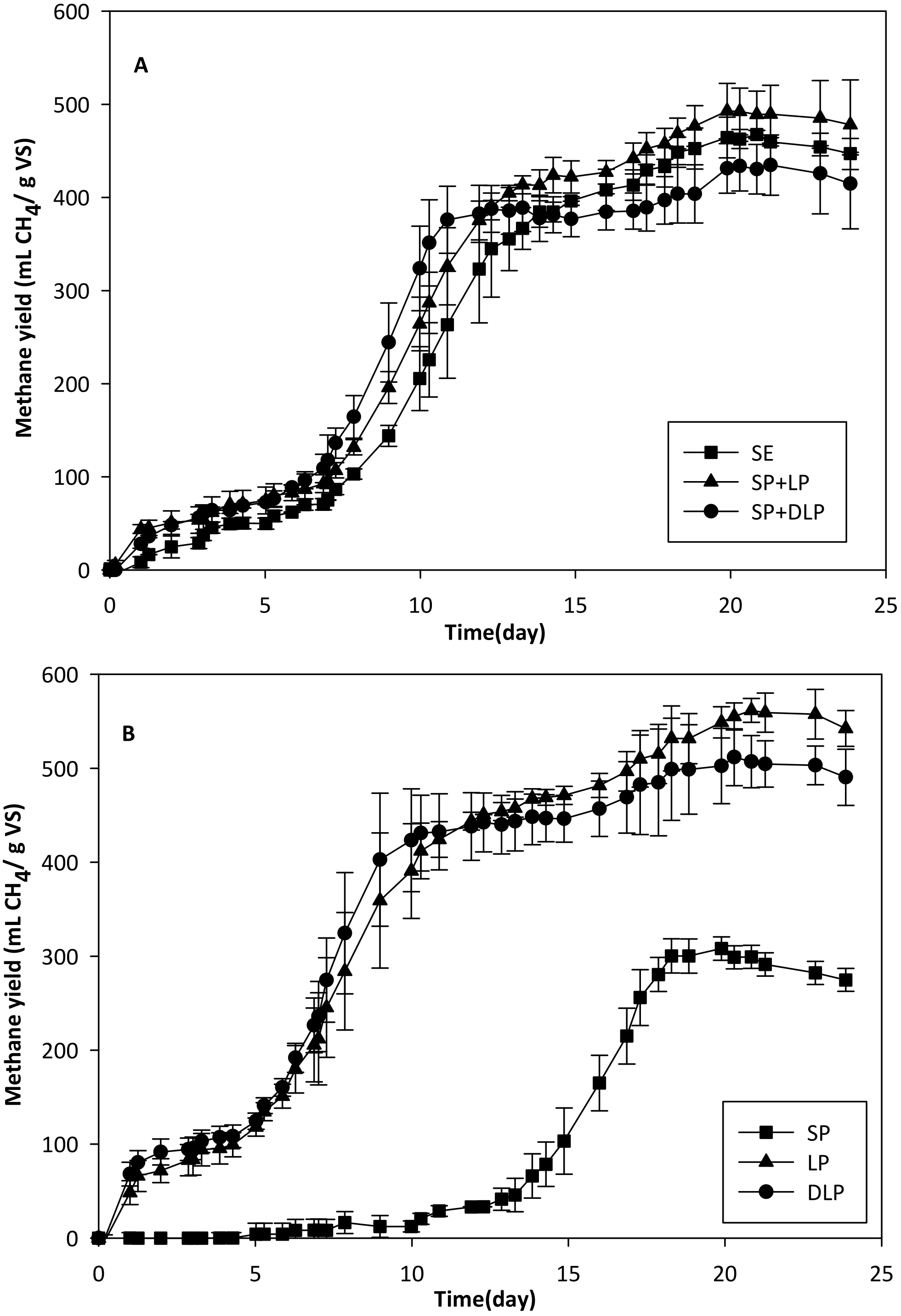

Figure 4.3. (**A**): Variation of cumulative methane production (mL CH_4/g VS) (VS, total volatile solids) with time for LP (liquid phase), SP (solid phase), and DLP (de-phenolized liquid phase) after 170 °C hydrothermal treatment with their standard deviations; (**B**):

Variation of cumulative methane production (mL CH_4/g VS) with time for the untreated SE (residual strawberry extrudate), SP + LP and SP + DLP mixtures after 170 °C hydrothermal treatment with their standard deviations.

Table 4.5. Values of the parameters obtained from the Logistic model (Sigmoidal 4 parameters) for the different substrates and mixtures studied (hydrothermal treatment at 220 °C).

Phases	***P*** **(mL CH_4/g VS)**	***R_{max}*** **(mL CH_4/g VS·d)**	***λ*** **(d)**	***B_0*** **(mL CH_4/g VS)**	**R^2**	**Error (%)**	**S.E.E.**
SE	429 ± 9	54.7 ± 6.3	10.6 ± 0.1	27 ± 6	0.9998	2.2	11.56
SP	310 ± 4	62.3 ± 4.9	15.72 ± 0.07	6 ± 1	0.9962	2.8	7.92
LP	552 ± 34	49.3 ± 9.3	7.8 ± 0.3	8.3 ± 1.5	0.9886	0.1	10.92
DLP	412 ± 17	68.2 ± 14.3	7.4 ± 0.1	70 ± 14	0.9838	5.7	11.10
SP + LP	431 ± 8	60.2 ± 8.6	10.0 ± 0.1	44 ± 6	0.9942	3.3	12.43
SP + DLP	346 ± 8	78.7 ± 12.8	8.7 ± 0.1	59 ± 7	0.9893	6.5	10.31

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5

Mesophilic semi-continuous anaerobic digestion of strawberry extrudate pre-treated with steam explosion

Abstract

The production of strawberry concentrate produces a side stream after extrusion that is commonly landfilled. This strawberry extrudate (SE), of lignocellulosic character, contains valuable bioactive compounds such as sugars and phenols. hydrothermal treatments, such as steam explosion, are currently used for the valorization of agricultural lignocellulosic wastes due to their ability to impact the structure of the lignocellulose and hemicellulose present in these wastes, favoring the disruption of fibers material. Steam explosion has already been shown as a promising technology for phenols recovery from SE. Biogas is an additional valuable resource that might be produced from hydrothermally pre-treated and de-phenolized SE. This study assessed the influence of a steam-explosion pre-treatment and the subsequent recovery of phenol compounds from the long-term operation of a semi-continuous anaerobic digester of pre-treated SE. The anaerobic digestion of SE steam exploded at 220 °C for 5 min and de-phenolized was stable at an OLR of 0.5 g of volatile solids (VS)/(L·d), which permitted a specific production rate of 135 ± 11 mL of CH_4/(g of VS d). The system was not able to operate at an OLR of 1 g of VS/(L·d), which resulted in a failure of the process. Despite the inhibition threshold of phenol compounds not being achieved, the inhibition of the anaerobic digestion process at an OLR of 1 g of VS/(L·d) was most likely due to the overloading of the system. This was indicated by the accumulation of soluble organic matter and volatile fatty acids. The increase in the propionic acid concentration up to 1300 mg/L when operating at OLRs higher than 0.5 g of VS/(L·d) could be the main factor responsible for the inhibition. An economic evaluation showed that the proposed approach (steam explosion, phenols recovery, and anaerobic digestion) would offer positive benefits, taking into account the high phenol recovery (0.90 g of gallic acid/kg of extrudate) and the low sales price of the phenols extract, i.e., EUR 0.610/g of gallic acid equivalents, needed to reach zero net profit.

✓ A modified version of this chapter has been published in *Foods* 9(12). (2020). 1887.

5.1. Introduction

Strawberries contain a wide variety of nutrients and phytochemicals, which are of great interest because they are beneficial for avoiding or preventing different cardiovascular, cancerous, and neurological diseases, among others (Giampieri et al. 2012; Martinsen et al. 2020). Given the excellent nutritional properties of the strawberry, most of the strawberries produced are distributed and sold in the fresh market. In recent years, an important part of this production has been the production of strawberry concentrate for products such as jams, yogurts, ice creams, juices, etc. (Martinsen et al. 2020). During the industrial process for obtaining strawberry concentrate, the strawberries are extruded through sieves with different mesh sizes, in such a way that a residual fraction, formed by the fibers part and the achenes, is retained, called the strawberry extrudate (SE) (**Chapter 2**). SE is, to some extent, used as animal feed, but most of it is landfilled (**Chapter 4**). Alternatives for the management of the SE are necessary to avoid severe environmental impacts caused by landfilling, such as negative effects on agricultural soil quality, atmospheric contamination, and/or the polluting of aquatic ecosystems.

Chapter 4 proposed the recovery of bioactive compounds from SE using heat treatments. These bioactive compounds can be used in the pharmaceutical, food, and/or cosmetic industries (Martinsen et al. 2020) (**Chapter 4**). The bioactive compounds from SE include ascorbic acid, anthocyanins, ellagitannins, different vitamins, carotenoids, folic acid, and flavonoids (Giampieri et al. 2012). Heat treatments move these compounds to a liquid phase, where they can be easily recovered (Rubio-Senent et al. 2013). One heat treatment widely used for lignocellulose material is steam explosion (Zheng et al. 2014). The steam-explosion treatment mainly affects the solubilization of the cellulose and lignin in the SE (Hendriks and Zeeman 2009; Zheng et al. 2014). Under steam-explosion conditions, the lignin is highly degraded, and the hemicellulose is hydrolyzed into its component sugars (**Chapter 2**). **Chapter 4** showed that the steam-explosion treatment of SE at 220 °C allowed the recovery of up to 2 g of gallic acid per kg of residual SE.

Chapter 4 also studied the anaerobic digestion of the steam-exploded and de-phenolized SE in batch mode. The anaerobic digestion of lignocellulosic materials, such as SE, is a promising alternative to valorizing these wastes because this treatment allows generating renewable and sustainable energy in the form of biogas (Kumar et al. 2018; Song et al. 2019). The pre-treatment of SE, such as steam explosion, could be a good complement for obtaining phenol compounds with health benefits and, at the same time, being able to improve their biodegradability by anaerobic digestion through the degradation of recalcitrant components (Carvalho et al. 2013; Guan et al. 2018) (**Chapter 3**).

The present work aimed to assess the long-term, semi-continuous mesophilic anaerobic digestion of a de-phenolized SE previously treated with steam explosion. Long-term, semi-continuous operation is crucial for studying process efficiency and reducing the risk of destabilization. The anaerobic digestion process was carried out over a long operational period (312 days), assessing different organic loading rates (OLRs), which were equivalent to several hydraulic retention times (HRTs).

5.2. Materials and methods

5.2.1. Strawberry extrudate and inoculum

The SE was provided by the company Hudisa S.A, located in Huelva, Spain (37.281813, −7.239095, Huelva, Spain). The SE was immediately stored at −20 °C after collection to avoid the uncontrolled fermentation of the substrate.

The anaerobic inoculum used in the anaerobic digestion process was obtained from the industrial anaerobic reactors located in the "Copero" wastewater treatment plant, located in Sevilla, Spain (37.309795, −5.986825, Dos Hermanas, Spain). The main chemical characteristics of the anaerobic inoculum were pH= 7.1 ± 0.1; alkalinity =4500 ± 65mg of $CaCO_3$/L; total solids (TS) = 21,100 ± 92 mg/L; volatile solids (VS) = 13,350 ± 194 mg/L; total chemical oxygen demand (COD) = 18,870 ± 630 mg of O_2/L; soluble COD (sCOD)=520 ± 8 mg of O_2/L; total phenols=100 ± 1mgof gallic acid/L; and total sugars = 20 ± 1 mg of glucose/L.

5.2.2. Steam-explosion treatment and extraction of phenol compounds

Steam explosion treatments were performed in a reactor at a 2-L capacity pilot-scale (Nusim, S.A., Madrid, Spain). The treatments with strawberry extrudate were carried out twice throughout the experiment, the first time 8.25 kg SE were treated and the second time 4.93 kg SE were processed. These were performed at a temperature of 220 °C and 25 kg/cm^2 pressure for 5 min. The amounts of steam consumed in both treatments were 6.71 and 2.64 kg steam/kg SE, respectively. After each treatment, the treated waste was centrifuged at 4700 g/1450 rpm (Centrifuge Comteifa, S. L., Barcelona, Spain) to separate a liquid phase (LP) and a solid phase (SP).

The extraction of the phenol compounds was carried out with a column, 4.5 cm in diameter and 140 cm in height, filled with 1L of adsorbent resin Amberlite XAD-16, which was described in **Chapter 4**. The Amberlite was dissolved in water, reaching a height of 120 cm in height in the column. Extractions were carried out from 10 L of liquid phase after hydrothermal treatment at 220 °C. The phase obtained after extraction has been called the de-phenolized liquid phase (DLP). The compounds adsorbed on the resin were extracted with 1 L EtOH 80 % (v/v) and 1 L EtOH 96 % according to Fernández-Bolaños et al (2011). The physicochemical characterization of untreated SE and different phases resulting from both steam explosion treatments and subsequent extraction of phenol compounds from SE is shown in **Table 5.1**. pressure for 5 min. An electronic computing device controlled the time and the temperature in a pre-programmed manner. After each treatment, the centrifuge (4226 Pacisa) was used to separate the liquid phase (LP) and solid phase (SP).

5.2.3. Anaerobic digestion set-up and experimental procedure

Anaerobic digestion reactors in triplicate were used. The three reactors were initially inoculated with 10 g of VS/L of inoculum. The reactors had a total capacity of 2.0 L, with a working volume of 1.7 L, and were continuously stirred by means of a stirrer (KMO 2 basic model, IKA-WERKE, Staufen, Germany). The operation temperature (35 °C) was maintained by a thermostatic chamber. The volume of methane was measured daily after CO_2 removal with tightly closed bubblers containing a 2N NaOH solution. The gas volume was measured by liquid displacement and expressed under standard temperature and pressure conditions (25 °C and 1 atm).

Table 5.1. Physicochemical characterization of the strawberry extrudate (SE) and of different phases resulting from hydrothermal treatment and extraction of phenol compounds.

	SE	**First steam explosion treatment**			**Second steam explosion treatment**		
		SP	**LP**	**DLP**	**SP**	**LP**	**DLP**
pH	3.7 ± 0.1	4.5 ± 0.1	4.0 ± 0.1	4.1 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.1
TS (g/kg SE)	142 ±1	97 ± 2	48 ± 1	40 ± 1	92 ± 2	45 ± 1	26 ± 1
VS (g/kg SE)	136 ± 1	93 ± 2	48 ± 1	40 ± 1	88 ± 2	41 ± 1	24 ± 1
COD (g O_2/kg SE)	200 ± 6	132 ± 2	61 ± 2	51 ± 2	171 ± 6	55 ± 6	34 ± 3
sCOD (g O_2/kg SE)	47 ± 1	9 ± 1	64 ± 2	54 ± 2	2 ± 1	51 ± 2	39 ± 1
Total phenols (g gallic acid/kg SE)	1.4 ± 0.1	0.7 ± 0.1	1.3 ± 0.1	0.4 ± 0.1	4.4 ± 0.1	0.5 ± 0.1	0.1 ± 0.1
Total sugars (g glucose/kg SE)	2.1 ± 0.1	0.5 ± 0.1	24.9 ± 0.1	21.8 ± 0.1	6.8 ± 0.1	25.6 ± 0.1	18.9 ± 0.1

The anaerobic digestion of the phases obtained after the steam-explosion treatments at 220 °C and subsequent extraction of the phenol compounds (SP + DLP) was carried out for 312 days, after an adaptation period of about 30 days, in which mixtures of a synthetic solution and SE were used (**Table 5.1**). The anaerobic digestion experimental study was carried out by using different organic load rates (OLRs), with three hydraulic retention times (HRTs) (**Table 5.2**). Additionally, the substrate resulting from the first steam-explosion treatment was used from the first day to the 230th day, while the SE from the second steam-explosion treatment was employed from the 231st day to the end of the study (Day 312).

Table 5.2. Experimental design.

Experimental Stage	Duration	Feeded Substrate	OLR (g VS/(L·d))
Bio-stimulation	16 days	SS	1
Adaptation	4 days	SS:SE 75:25, in VS	1
	13 days	SS:SE 50:50, in VS	1
	12 days	SE 100, in VS	1
Stage 1	56 days (0-56)	SP+DLP	1
Stage 2	50 days (57-107)	SP+DLP	0.5
Stage 3	13 days (108-121)	SP+DLP	1
Stage 4	62 days (122-284)	SP+DLP	0.75
Stage 5	26 days (285-312)	SP+DLP	1

SS, Synthetic solution with glucose (50 g/L) and sodium acetate (25.2 g/L).

5.2.4. Chemical analyses

To measure the water-soluble compounds of the SE and the solid phase (SP) after the steam-explosion treatments, an extraction process was carried out as explained in **Chapter 4**. To extract the soluble compounds from the liquid phase (LP), de-phenolized liquid phase (DLP), and effluents of the reactors, the samples were centrifuged and microfiltered with 0.45 µm nylon microfilters.

The pH, TS, VS, and alkalinity were analysed according to the standard methods of the American Public Health Association (APHA, (2017)). The total chemical oxygen demand (COD; mg of O_2/L) was measured as explained in **Chapter 4**; the colorimetric standard method 5220D was used for the measurement of the soluble COD (sCOD; mg of O_2/L) (APHA 2017). Total phenols were measured, after extraction with a methanol/water solution (80:20) at 70 °C and subsequent filtration through 0.45 µm filters, by the Folin–Ciocalteu spectrophotometric method and expressed as mg of gallic acid equivalents/L (Singleton and Rossi 1965; García et al. 2016). Total sugars were measured by the anthrone colorimetric method, using a spectrophotometer (Biorad iMark Microplate Reader, Hercules, CA, USA), and expressed as mg of glucose equivalents/L (Mokrash, L. 1954). The determination of volatile fatty acids (VFAs) was carried out using gas chromatography. More details about the VFA determination can be found in **Chapter 6**.

5.2.5. Economic assessment

An economic evaluation was carried out to calculate the minimum sale price of the extracted phenols that allows a positive economic balance. The case studied includes steam-explosion treatment, phenols extraction, and anaerobic digestion with the most stable OLR. The generated biogas is used for the simultaneous generation of electricity and heat. The net benefit of the different options was defined as the economic balance between the operational costs and income from sales. The minimum cost for the sale of the phenols extracted was calculated by imposing a value of zero on the net benefit and was expressed in EUR/g of gallic acid equivalents. The phenols extraction efficiency was 64 %, which was obtained from the reduction of gallic acid observed in the SE during the experiments and its increase in the liquid phase after the extraction process with respect to untreated SE. The energy production was based on the methane production for Stage 2, i.e., 57–107 days and OLR = 0.5 g of VS/(L·d) (**Table 5.2**), and the use of a cogeneration biogas engine for electricity and hydrothermal energy. The energy consumption for the steam explosion was based on the steam requirement, i.e., 6.71 kg of steam/kg of SE. More details about the previous considerations for the economic assessment can be found in **Chapter 3**.

5.3. Results and discussion

5.3.1. Methane production rate at different organic loading rates throughout the experimental period

Figure 5.1. shows the variation of the daily methane production rate throughout the digestion time for the different OLRs tested. **Table 5.3** summarizes the pH, alkalinity, TS, VS, sCOD, total VFA, and total phenols of the effluents of the semi-continuous anaerobic digestion process for the steam-exploded SE, as well as the methane production rates, and biodegradability achieved for the different organic loading rates (OLRs) tested over the five operational periods carried out. During the first 20 days, a methane production rate higher than 250 mL of CH_4/(g of VS·d) was observed, a value that was not achieved again throughout the experiment. After these first 20 days, the reactors were destabilized, exhibiting a significant drop in the methane production rate. In Stage 1, i.e., 0–56 days and OLR = 1 g of VS/(L·d), the methane production rate presented a mean value of around 124 ± 46 mL of CH_4/(g of VS d) and a biodegradability of 70 % (**Table 5.3**). Given that the methane production rate was unstable in Stage 1 at

OLR = 1 g of VS/(L·d), it was decided to decrease the organic loading rate to a value of 0.5 g of VS/(L·d). In Stage 2, i.e., 57–107 days and OLR = 0.5 g of VS/(L·d), the methane production rate observed was more stable, reaching a mean value of around 135 ± 11 mL of CH_4/(g of VS·d), and the biodegradability was 53 %. It can be seen that the biodegradability was 20 % lower than that achieved in Stage 1, because the reactors were more stabilized, and they had been accumulating solids with low biodegradability since the beginning of the experiment. In Stage 3, i.e., 108–121 days, it was decided to again raise the OLR to 1 g of VS/(L·d) since in Stage 2, the reactor was stabilized. In Stage 3, the methane production rate presented a mean value of around 130 ± 10 mL of CH_4/g of VS d and a biodegradability of 51 %, which were very similar to those achieved in Stage 2. As can be seen in **Figure 5.1**, the reactors could not withstand an OLR = 1 g of VS/(L·d), so it was decided not to maintain this OLR over an operational period equivalent to three times the HRT and to quickly change to a lower load before the reactors could destabilize. In Stage 4, i.e., 122–284 days and OLR = 0.75 g of VS/(L·d), the methane production rate showed a mean value of around 129 ± 45 mL of CH_4/g of VS d, and the biodegradability was 60 %. In this Stage 4, it was decided to maintain this load for a longer time for the study of the stability of the OLR, and it was observed that the stability could only be maintained when the reactors were supplemented with alkalinity. Finally, in Stage 5, i.e., 285–310 days, the OLR was again augmented up to OLR = 1 g of VS/L d to finish observing that at this load, the reactors were not stable; the methane production rate presented a mean value of around 64 ± 43 mL of CH_4/g of VS d, and the biodegradability was 48 %. The characteristics of the substrate described in **Table 1** indicate that the acidification of the reactors could occur, and therefore, an inhibition of methane production, due to the low pH value and the high sugar content, could take place (Kryvoruchko et al. 2009). Due to the instability observed in the methane production rates throughout the entire experimental period of the semi-continuous anaerobic digestion process, the highest methane production achieved in this process was lower than that achieved in the batch anaerobic digestion experiments for this steam-exploded waste after 24 days of digestion time, which was 430 mL of CH_4/g of VS (**Chapter 4**). Siles et al. (2013) reported that the methane production in the anaerobic digestion of strawberry extrudate, with similar conditions but without pre-treatment, was 230 mL of CH_4/g of VS at OLRs = 0.73 and 1.07 g of VS/(L·d). In another study, untreated strawberry residue obtained from rejections provided by a supermarket yielded a methane production rate of 231 mL of CH_4/g of VS at an OLR = 4.4 g of VS/(L·d) (Arhoun et al. 2017). Finally, a recent study of the anaerobic digestion of strawberry waste at a pH controlled at 7 reported a methane production of 353 ± 10 mL of CH_4/g of VS (Serrano et al. 2020), which was higher than the rates reported in the above-mentioned studies.

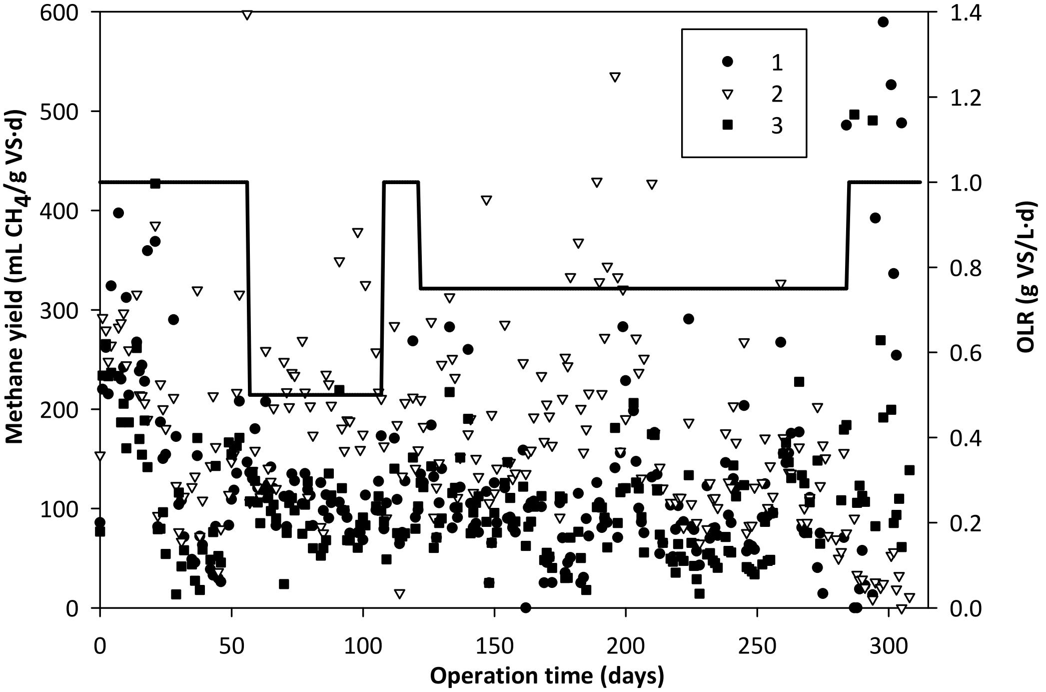

Figure 5.1. Variation of the methane production rates and organic loading rates (OLR, line bold) during the whole experimental time.

Table 5.3. pH, alkalinity, TS, VS, sCOD, total VFA, total phenols, methane production rates and biodegradability during the different organic loading rates (OLRs).

	Stage 1	**Stage 2**	**Stage 3**	**Stage 4**	**Stage 5**
OLR (g VS/L·d)	1	0.5	1	0.75	1
Days	0-56	57-107	108-121	122-284	285-310
pH	6.3 ± 0.6	6.1 ± 0.3	6.2 ± 0.3	6.0 ± 0.7	6.9 ± 0.5
Alkalinity (mg $CaCO_3$/L)	1500 ± 660	915 ± 177	1172 ± 281	1717 ± 237	8032 ± 333
TS (mg/L)	9588 ± 2840	10842 ± 866	12746 ± 1318	14990 ± 4963	26163 ± 2636
VS (mg/L)	7658 ± 2673	9412 ± 950	11200 ± 1541	12848 ± 3454	15581 ± 324
sCOD (mg O_2/L)	1799 ± 1394	2372 ± 165	2793 ± 194	3741 ± 1504	12108 ± 1412
Total VFA (mg O_2/L)	1838 ± 1752	2139 ± 255	2418 ± 219	3131 ± 893	5200 ± 8
Total phenols (mg gallic acid/L)	63 ± 27	119 ± 22	119 ± 58	182 ± 63	271 ± 37
Methane production (mL CH_4/g VS·d)	124 ± 46	135 ± 11	130 ± 10	129 ± 45	64 ± 43
Biodegradability CH_4 (%)	10 ± 3	9 ± 2	16 ± 1	12 ± 4	7 ± 2
Biodegradability VS (%)	70 ± 4	53 ± 1	51 ± 1	60 ± 10	48 ± 9

5.3.2. Stability and organic matter soluble concentration throughout the experimental time

Throughout the experimental time, the pH, alkalinity (mg of $CaCO_3$/L), sCOD (mg of O_2/L), and total VFAs (mg of O_2/L) were determined to evaluate the process stability and the variation of organic matter with time in the semi-continuous anaerobic digestion process (**Figure 5.2**). As can be seen, the pH was only stabilized at an OLR = 0.5 g of VS/(L·d), in Stage 2. At OLRs higher than 0.5 g of VS/(L·d), the pH began to decrease, due to the pH of the added load always being less than 4.5 (**Table 5.2**). Therefore, for OLRs greater than 0.5 g of VS/(L·d), the alkalinity must be increased so that the reactors can buffer changes in pH. It can be seen in Stages 1 and 4 that the pH tended to decrease from pH 7.6–7.8, which can be considered as the optimal pH (Wheatley 1990), to an acidification value that reached around pH 5. A decrease in pH to 5.8 was also reported by Arhoun et al. (2017) in the anaerobic digestion of strawberry residues. These authors also described that at low pH, the methane concentration also decreased and, finally, the biological activity ceased, as observed in the last stage of their study. **Figure 5.2** shows how the alkalinity decreased when the pH dropped and increased again when the sodium bicarbonate buffer was added to the reactors. The decreasing trend varied from 3000 mg of $CaCO_3$/L to 500 mg of $CaCO_3$/L in the different stages. As was demonstrated throughout the experiment, buffer must be added for this type of substrate to avoid reactor acidification, given its acidic character. The values of the alkalinity in the reactors were below 3000 throughout the experiment, while it is recommended to achieve values of around 5000 to ensure the adequate stability of the process, as reported in other studies (Serrano et al. 2019).

Figure 5.2 also shows the sCOD and total VFA variation throughout the digestion time for the different OLRs assayed. The sCOD and VFAs are important parameters in the anaerobic digestion processes for indicating the performance of the hydrolysis/acidogenesis stages of these processes (Lu et al. 2018). In stage 1, i.e., 0–56 days and OLR = 1 g of VS/(L·d), the sCOD and total VFAs increased to values of 4000 mg of O_2/L. This high increase in sCOD and total VFAs caused a decrease in pH and alkalinity, and thus, in turn, methane production was unstable in Stage 1 with OLR = 1 g of VS/(L·d). Therefore, it was decided to decrease the organic loading rate to a value of 0.5 g of VS/(L·d). In Stage 2, i.e., 57–107 days and OLR = 0.5 g of VS/(L·d), sCOD and total VFAs showed more stable values, reaching mean values of around 2372 ± 165 mg of O_2/L and 2139 ± 255 mg of O_2/L, respectively. In Stage 3, i.e., 108–121 days, the OLR was again increased to 1 g of VS/(L·d) since in Stage 2, the reactor was stabilized, resulting in sCOD and total VFA mean values of around 2793 ± 194 mg of O_2/L and 2418 ± 219 mg of O_2/L, respectively, very similar to those reached in Stage 2. As can be seen in **Figure 5.2**, the pH and alkalinity decreased again, and the reactors did not support an OLR = 1 g of

VS/(L·d), so it was decided not to wait for three times the HRT with this OLR and to quickly change to a lower load before the reactors destabilized. In Stage 4, i.e., 122–284 days and OLR = 0.75 g of VS/(L·d), the sCOD and total VFAs presented mean values of around 3741 ± 1504 mg of O_2/L and 3131 ± 893 mg of O_2/L, respectively. In Stage 4, it was decided to maintain the mentioned OLR for a longer time to assess the process stability. In this case, it was observed that it was necessary to provide a buffer for keeping the stability. Finally, in Stage 5, i.e., 285–310 days, the OLR was again increased to 1 g of VS/(L·d) to finish the study. It was observed that at this load, the reactors were not stable; the sCOD and total VFAs presented mean values of around 12,108 ± 1412 mg of O_2/L and 5200 ± 8 mg of O_2/L, respectively. The increases in COD and VFAs were produced by the overloading and saturation of the reactors when exceeding the OLR limit. A similar trend was observed when the OLR increased from 1 to 2 g of VS/(L·d) in the semi-continuous anaerobic digestion of olive mill solid waste (Serrano et al., 2019). **Table 5.3** also shows that in all the stages, more than 80 % of the total solids corresponded to VS, except in the last stage, in which this value was only 60 % of the VS.

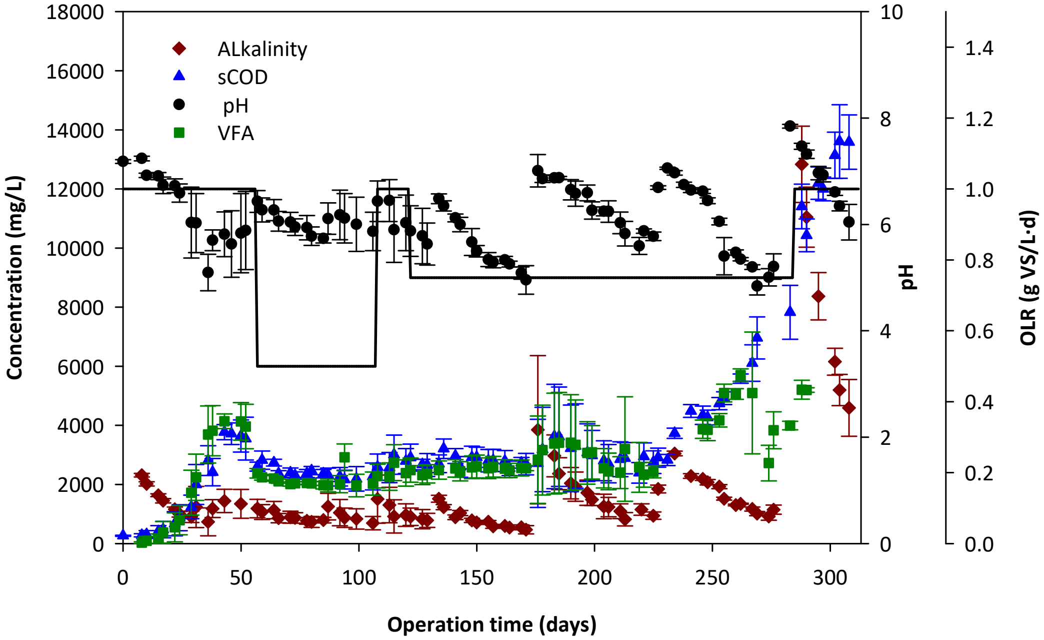

Figure 5.2. Variation of pH (●), Alkalinity (◆), sCOD (▲) and VFA (■) values with their standard deviations throughout the experimental period for the different organic loading rates (OLR, line bold) assayed.

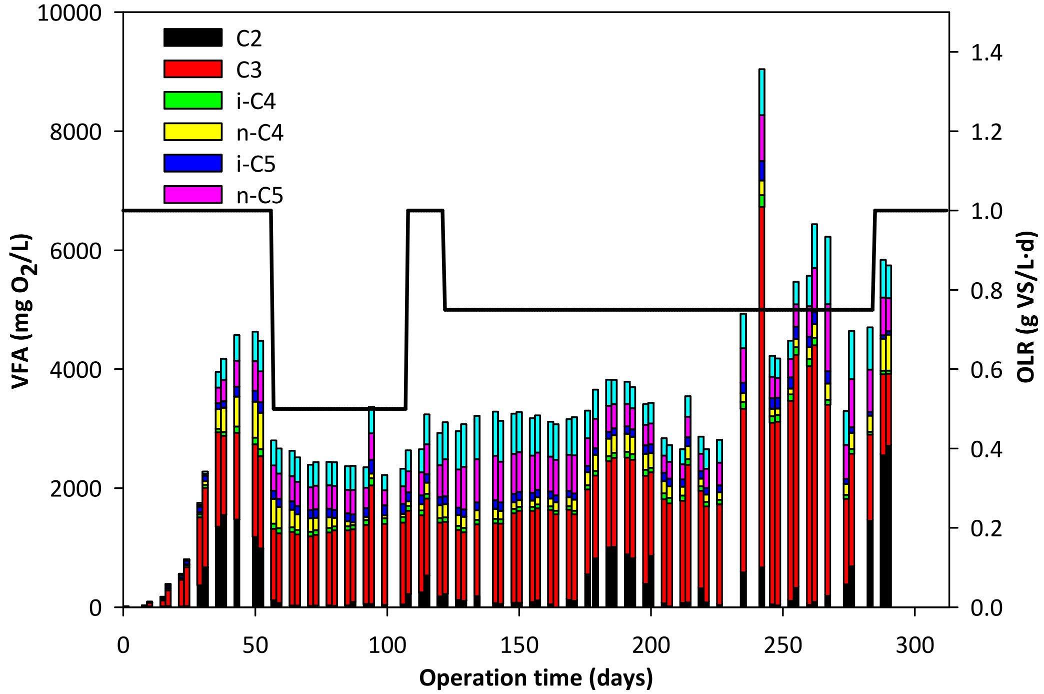

Figure 5.3. Variation of the VFA values, with their standard deviations, under the different organic loading rates (OLRs, line bold) during the experimental time.

5.3.3. Variation of the concentration of phenol compounds with organic loading rate throughout the experimental time

Phenol compounds were determined once per week and are expressed in mg of gallic acid/L (**Figure 5.4**). The concentration of phenol compounds increased in Stage 1 and at the beginning of Stage 2 until reaching an average value of 119 ± 22 mg of gallic acid/L.

As shown in **Figure 5.4**, the phenol compounds remained approximately constant until the end of Stage 4, where they began to increase again due to the instability of the reactors. The highest concentration of phenol compounds was reached in Stage 5, with 271 ± 37 mg of gallic acid/L, when the reactors are unstable due to the excess of added organic load (**Figure 5.4**). Phenol compounds at a certain concentration can inhibit the activity of anaerobic microorganisms, affecting the microbial growth (Borja et al. 1997; Battista et al. 2015). However, the phenol concentrations found in these assays were always lower than those considered inhibitory for anaerobic digestion processes. The increase in phenols was similar to that found by Serrano et al. (2020), who reported that exploiting the high solubilization capacity of phenols in an acidogenic fermentation at pH 4 is a promising method for the recovery of phenols from strawberry waste (Serrano et al. 2020).

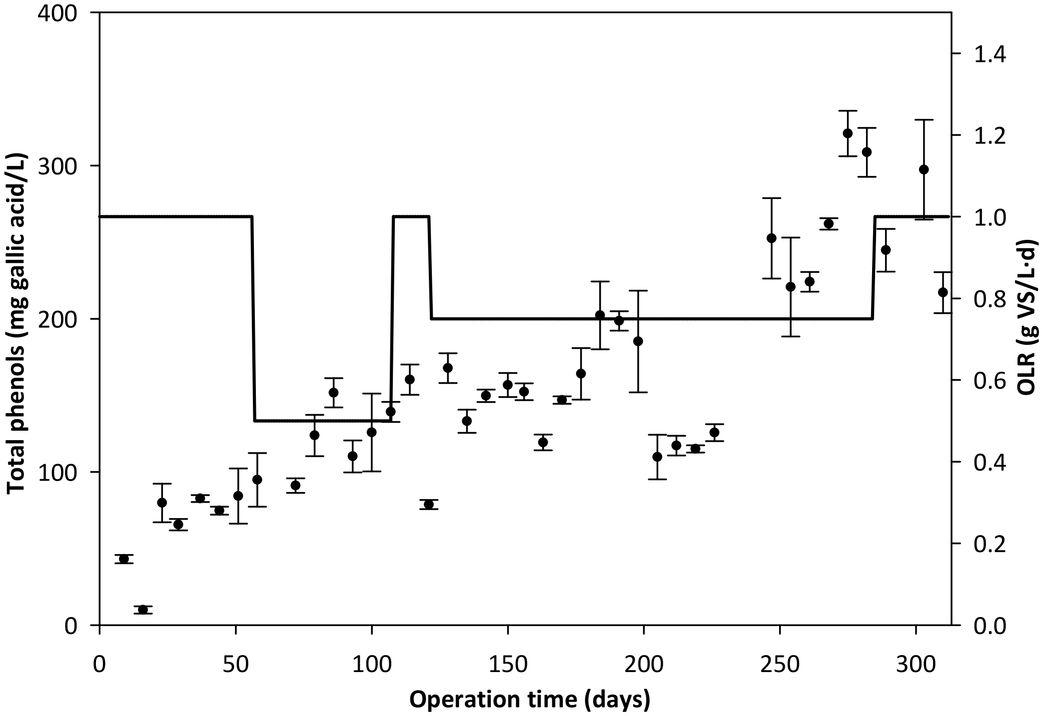

Figure 4. Variation of the total phenols concentrations, with their standard deviations, with the organic loading rate (OLR, bold line) throughout the experimental time.

5.3.4. Economic assessment

The minimum sales price of the phenol compound extract was calculated for zero net profit, being EUR 0.610/g of gallic acid equivalents for economic viability at an OLR of 0.5 g of VS/(L·d). In a recent study in **Chapter 3**, in which two strawberry extrudates from two different campaigns were hydrothermally treated at 150 °C for 60 min, similar results were obtained, i.e., EUR 0.556 and 1.23/g of gallic acid equivalents. However, the steam consumed in the steam-explosion treatment of SE, 6.71 kg of steam/kg of SE, was much higher than the values reported in the study in **Chapter 3**, i.e., 1.93 and 0.44 kg of steam/kg of SE. Moreover, the extracted phenol compounds in both studies were also similar, 0.90 g of gallic acid/kg of extrudate from steam-exploded SE in the present work versus 0.88 g of gallic acid/kg of extrudate in Strawberry Extrudate 1 in the study in **Chapter 3**. According to these data, the high steam consumption was not compensated for by the low methane production yield at an OLR of 0.5 g of VS/(L·d); however, the high recovery and a low sales price for the phenol compounds made the economic assessment positive.

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6

Valorization of strawberry extrudate waste: recovery of phenol compounds by high-temperature hydrothermal treatment and methane production by mesophilic semi-continuous anaerobic digestion

Abstract

A biorefinery option for the valorization of the waste generated in the processing of strawberry by-products called strawberry extrudate (SE) was carried out in this study. Firstly, it was been proposed to obtain high added value compounds, such as phenols either in strawberries, and in strawberry extrudate generated in the strawberry industry. A high temperature hydrothermal treatment was carried out for the solubilization of the phenol compounds at a temperature of 170 °C during 60 min. After the hydrothermal pre-treatment, the phenols were extracted with an adsorbent resin known as Amberlite XAD 16, extracting 0.6 ± 0.1 g gallic acid/kg strawberry extrudate. Finally, the biomass remaining after the extraction of the phenol compounds was subjected to an anaerobic digestion process. The anaerobic digestion of pre-treated strawberry extrudate achieved a stable methane production value of 243 ± 34 mL CH_4/(g volatile solids (VS)·d) at an organic loading rate of 1.25 g VS /(L·d). An economic balance was also been made to obtain an economically positive value of the strawberry extrudate, obtaining that the minimal price of the phenol extract for having benefits from the proposed biorefinery concept was EUR 0.812/g of gallic acid equivalents.

6.1. Introduction

In the last ten years, world strawberry production has increased from 6 to almost 9 million tons (FAO 2021). Strawberries provide a wide range of phenol compounds, high content of fibers, micronutrients and ascorbic acid (Giampieri et al. 2012) (**Chapter 1**). Consequently, these customer-friendly and health-supporting properties have led to the solid economic role of the strawberry, with a global strawberry market-value exceeding US$ 23,600 million per year (FAO 2021; Warner et al. 2021). This also leads to an increase in the wastes generated by the strawberry industry, mainly those generated in the manufacture of by-products such as jams, yogurts and/or ice creams, called strawberry extrudate (SE) (**Chapter 1**). The increase in the generation of SE would be negatively associated with the cost derived from the need for an adequate treatment to avoid environmental impacts of this easily putrefiable substrate. However, SE similar to strawberries has different high-value compounds, that could be interesting of being extracted (**Chapter 1**).

Different hydrothermal treatments have been proposed and applied for SE to facilitate the extraction of high added value compounds from liquid phase after centrifugation (Rubio-Senent et al. 2012) (**Chapter 3**). The lignocellulosic material contained in the SE possess a large amount of phenol compounds in addition to a large amount of soluble sugars, as it was recently reported in previous studies (Millati et al. 2020) (**Chapter 2**). After the hydrothermal treatment, the recovery by extraction of the solubilized desirable compounds, such as the phenol compounds, has been reported to be done by adsorption with an adsorbent resin (Chavan and Gaikwad 2021; Li et al. 2021; Neggad et al. 2021). As an example, the use of resin Amberlite XAD 16 achieved an extraction of around 50 % of phenol compounds contained in the SE hydrothermal pre-treated (**Chapter 4**).

The extraction and recovery of the phenol compounds contained in SE, which are well-Known microbial inhibitors, could be beneficial for a subsequent biological management of the remaining biomass (Rashama et al. 2021). Anaerobic digestion process of agro-waste is a well-stablished biotechnology that is commonly used to obtain energy from the methane produced (Yan et al. 2017). Different previous studies of anaerobic digestion of diverse agro-wastes have been carried out, observing how the pre-treatment proposed in this study allowed improving the methane production that can be obtained from these residues (Serrano et al. 2019a, b; Ahmed et al. 2021) (**Chapter 3**). The increase in the methane production derived of the hydrothermal treatments is usually related to the solubilization of the lignocellulosic biomass through hydrothermal pre-treatments carried out at temperatures above 120-180 °C (Serrano et al. 2019b; Bhatia et al. 2021). The hydrothermal pre-treatment of the SE at these temperatures should favor its subsequent anaerobic digestion since the bonds between cellulose, hemicellulose and lignin could be disintegrated, thus improving the accessibility of hydrolytic enzymes to the released polysaccharides (Yadav et al. 2019). However, highly severe conditions during the hydrothermal treatments should be avoided due to the formation of refractory compounds for biogas production as was observed in the other studies (Serrano et al. 2019b; Liao et al. 2021; Ghimire et al. 2021). In a study carried out in **Chapter 4** it was observed that with hydrothermal pre-treatments at 170 °C during 60 minutes and 220 °C during 5 minutes and a subsequent extraction of phenol compounds, biochemical methane potential (BMP) tests allowed observing how rate of methane production increased with respect to the untreated SE. In another recent study, the same substrate was used to perform a semi-continuous anaerobic digestion test after the pre-treatment of the waste at 220 °C during 5 minutes and a subsequent extraction of phenol compounds, observing that the reactors were unstable, despite what was seen in the BMP tests. A low methane production of 135 ± 11 mL of CH_4/(g VS·d) (VS, volatile solids) for an low organic loading rate (OLR) of 0.5 g VS/(L·d), observing that the pH and alkalinity were not stable and decreased with increasing OLR (**Chapter 5**). This indicates that long-term operation is influenced by certain factors, such as the accumulation of inhibitors, which were not detected in simpler and more specific studies, such as BMP test (**Chapter 4**). Therefore, it is necessary to evaluate pre-treatment conditions less severe than 220 °C to see the methane production is more stable.

Therefore, the objective of this work was to analyze the valorization of SE through a combination of phenol compounds extraction and semi-continuous anaerobic digestion process. To achieve this objective, it was proposed to carry out a hydrothermal pre-treatment at 170 °C for 60 minutes, recovering phenol compounds with the adsorbent resin Amberlite XAD 16. Finally, the remaining biomass was subjected to a mesophilic semi-continuous anaerobic digestion during a long term period of 273 days with different organic loading rates.

6.2. Materials and methods

6.2.1. Pre-treatment of strawberry extrudate: High-hydrothermal treatment and extraction of phenol compounds

The sample of strawberry extrudate used in this study was described in detail in **Chapter 5**. High-temperature hydrothermal treatments have been carried out at a temperature of 170 °C and 8.7 kg/cm^2 pressure for 60 min using a steam treatment reactor described in **Chapter 4**. The treatment of the SE was carried out twice throughout the experimental time, the first time 10.06 kg of SE were treated with a steam consumption of 1.03 kg steam/kg SE. The second time, 4.93 kg of SE were treated and 2.22 kg steam/kg SE were consumed. After each treatment, and to promote the recovery of phenol compounds the treated SE was centrifuged at 4700 g/1450 rpm (Centrifuge Comteifa, S. L., Barcelona, Spain) for separating a liquid phase (LP) and a solid phase (SP). The phenol compounds were extracted from the LP using the adsorbent resin Amberlite XAD-16, obtaining a de-phenolized liquid phase (DLP) by the method described in **Chapter 4**. The physicochemical characterization of different samples resulting from both hydrothermal treatments, extraction of phenol compounds, and SE without treatment are shown in **Table 6.1**.

Table 6.1. Physicochemical Characterization of different phases resulting from untreated and pre-treated SE.

		First hydrothermal treatment			**Second hydrothermal treatment**		
	SE	**SP**	**LP**	**DLP**	**SP**	**LP**	**DLP**
pH	3.7 ± 0.1	4.1 ± 0.1	3.9 ± 0.1	4.0 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	4.0 ± 0.1
TS (g/kg SE)	142 ±1	122 ± 3	36 ± 1	27 ± 1	82 ± 2	48 ± 1	46 ± 1
VS (g/kg SE)	136 ± 1	117 ± 3	33 ± 1	27 ± 1	79 ± 1	45 ± 1	41 ± 1
COD (g O_2/kg SE)	200 ± 6	173 ± 1	49 ± 1	35 ± 1	108 ± 2	57 ± 0	46 ± 1
sCOD (g O_2/kg SE)	47 ± 1	27 ± 1	45 ± 1	38 ± 1	3 ± 1	56 ± 1	47 ± 1
Total phenols (g gallic acid/kg SE)	1.4 ± 0.1	2.2 ± 0.1	1.0 ± 0.1	0.4 ± 0.1	1.4 ± 0.1	2.6 ± 0.1	0.9 ± 0.1
Total sugars (g glucose/kg SE)	2.1 ± 0.1	9.0 ± 0.1	18.7 ± 0.1	16.1 ± 0.1	3.9 ± 0.1	21.6 ± 0.1	18.1 ± 0.1

6.2.2. Experimental procedure of mesophilic semi-continuous anaerobic digestion

The anaerobic inoculum used in this study was obtained in the "Copero" wastewater treatment plant, located in Sevilla, Spain. More details about the inoculum characterization can be found in **Chapter 5.** Three glass reactors of 2.0 L total volume were operated in anaerobic conditions, with a final working volume of 1.7 L. The reactors were inoculated with anaerobic biomass at a concentration of 10 g VS /L. The reactors were continuously stirring through a cylindrical magnetic bar and every day samples of the reactors were taken and SE was fed with a syringe of 100 mL capacity. The volume of methane was measured after CO_2 removing with a NaOH solution (3 N) in tightly bubblers. Methane production was expressed at standard temperature and pressure conditions (25 °C and 1 atm).

During the first 45 days, the three reactors were conditioned and acclimated with mixtures of a synthetic solution (SS) with glucose (50 g/L) and sodium acetate (25.2 g/L) and SE untreated as described in **Table 6.2**. After adaption, the reactors were operated during 273 days and fed with SP+DLP after realized pre-treatments, using different OLRs, during at least three hydraulic retention times (HRTs). Sample of second hydrothermal treatment was used from the 226 days up to the end of the experimental time (day 273).

Table 6.2. Anaerobic digestion set-up and experimental design.

Experimental Stage	Duration	Feeded Substrate	OLR (g VS/(L·d))
Bio-stimulation	16 days	SS	1
Adaptation	4 days	SS:SE 75:25, in VS	1
	13 days	SS:SE 50:50, in VS	1
	12 days	SE 100, in VS	1
Stage 1	78 days (0-77)	SP+DLP	1
Stage 2	21 days (78-98)	SP+DLP	1.5
Stage 3	76 days (99-174)	SP+DLP	1
Stage 4	73 days (175-247)	SP+DLP	1.25
Stage 5	26 days (248-273)	SP+DLP	1.5

SS, Synthetic solution with glucose (50 g/L) and sodium acetate (25.2 g/L).

The calculation of biodegradability in methane was carried out from the grams of volatile solids added each day and the theoretical methane per grams of volatile solids at standard temperature and pressure conditions (25 °C and 1 atm) using the following (**Equation (1)**). The theoretical methane production was calculated from the total chemical oxygen demand of the SP and DLP (**Table 6.1**) with a conversion factor g O_2/g VS of 1.42, i.e., 382 ml CH_4/g VS.

$$Biodegradability\ CH_4(\%) = \frac{g_{added}\ VS\ x\ 382\ ml\frac{CH_4}{g\ VS}}{experimental\ ml\ CH_4} x100 \quad \textbf{Equation (1)}$$

The calculation of the biodegradability in volatile solids was carried out by dividing the grams of volatile solids added each day between the grams of volatile solids inside the reactor (**Equation (2)**).

$$Biodegradability\, VS(\,\%) = \frac{g_{reactor}\, VS}{g_{added}\, VS} x100$$ **Equation (2)**

6.2.3. Chemical analyses

To measure the water-soluble compounds of the SE and the solid phase (SP), 160 g of distilled water were added to 20 g of sample and keeping it stirred for 24 h (Thompson et al. 2002). Soluble compounds from samples were centrifuged and microfiltered with 0.45 µm nylon microfilters.

pH, TS total solids (TS), volatile solids (VS), alkalinity, soluble chemical oxygen demand (sCOD) were measured in the samples according to the Standard Methods (APHA 2017). Chemical oxygen demand (COD), total phenols, and total sugars were measured in different samples as described previously in **Chapter 2**. Volatile fatty acids (VFA) (C2-C5) were determined is carried out using a Shimadzu GC-2010 gas chromatograph equipped with a column of 100 % ethylene glycol composition of 0.25 mm x 25 m and a flame ionization detector (FID). The oven temperature gradually increases from 100 to 170 ° C at a rate of 5 ° C/min. Nitrogen (30 mL/min), hydrogen (40 mL/min), and air (399.8 mL/min) were used as carrier gas at a flow rate of 40.1 mL/min at 456 kPa. Determined individual VFA concentrations were expressed as COD by using the stoichiometric COD factor of 1.0667, 1.512, 1.813, 1.813, 2.036, and 2.036, for acetic, propionic, butyric, iso-butyric, valeric, and iso-valeric, respectively (Paulose and Kaparaju 2021). All measurements were made in triplicate to obtain mean and standard deviation values.

6.2.4. Economic assessment

With the aim of predicting if the SE valorization proposed in this study is a good option, an economic valuation was carried out for a scenario including the pre-treatment of the SE with the high-temperature hydrothermal treatment followed by the extraction of phenol compounds and an anaerobic digestion process with the most stable OLR achieved in the experimental procedure. The cogeneration of thermal energy and electricity from the generated biogas was also proposed for the valorization of the anaerobic digestion process. The calculation of the net benefit for the proposed scenario and the minimum cost for the sale of the phenols extracted were described in detail in **Chapter 5**. The phenol extraction efficiency on the untreated SE was 43 % in the first hydrothermal treatment (**Table 6.1**). The energy cogeneration production was based on the methane production from the Stage 4 of the present study, i.e., 175-247 days and the

OLR = 1.25 g of VS/(L·d) (**Table 6.2**). The energy consumption for the high-hydrothermal treatment was based on the steam requirement, i.e., 1.03 kg of steam/kg of SE. More details for the economic assessment can be found in **Chapters 3 and 5.**

6.3. Results and discussion

6.3.1. Daily methane production along the experimental period

The mixture SP+DLP obtained after hydrothermal treatment and subsequent extraction of phenols from SE was evaluated through the variation of the daily methane production rate with time (**Figure 6.1**). As can be seen, the anaerobic digestion process has showed a fluctuating methane production trend with the changed OLR. In the first stage, i.e., 0–77 days and OLR = 1 g VS/(L·d), the methane production rate presented a mean value of 216 ± 26 mL CH_4/(g VS·d) and a biodegradability in VS around 70 % (**Table 6.2**). The production was lower than that obtained in a BMP test form SE with the most optimal conditions, and with the same pre-treatment, obtaining a methane production of 430 mL CH_4/g VS (**Chapter 4**). However, this methane production rate value at OLR of 1 g VS/(L·d) was 21 % higher than that achieved for the olive mill solid waste treated with the same conditions of hydrothermal treatment, extraction of phenols and anaerobic digestion process (Serrano et al. 2019b). When the OLR was increased to 1.5 g VS/(L·d), between days 78-98, an inhibition in the methane production process was observed (**Figure 6.1**), which presented an average value of around 66 ± 26 mL CH_4/(g VS·d) and a biodegradability of 61 %. Also, an increase in solid concentrations (TS and VS) was also observed due to the decrease in the percentage of degradation (**Table 6.3**). In the third stage, between days 99–174 days and again at an OLR = 1 g VS/(L·d), the methane production rate was recovered, achieving a mean value of 253 ± 26 mL CH_4/(g VS·d) and a biodegradability of around 67 %. This higher conversion rate from SE to methane may be a direct consequence of the higher availability of organic matter for the same inoculum, that is, a higher ratio of inoculum to substrate at an OLR of 1 g VS/(L·d) in comparison with this ratio at an OLR of 1.5 g VS/(L·d) (Ziaee et al. 2021). With the increase in OLR this time to 1.25 g VS/(L·d) at days 175-247, a similar methane production rate and biodegradability were observed (**Table 6.2**), which presented an average value of around 243 ± 34 mL CH_4/(g VS·d) and 69 %, respectively. Finally, in the stage 5, when the OLR of reactors were increased again from 1.0 to 1.5 g VS/(L·d), days 248-273, an inhibition was observed in the methane production (**Figure 6.1**), i.e., methane production rate rapidly dropped up which presented values less than 20 mL CH_4/(g VS·d) and a biodegradability around of 58 % up to the complete inhibition the reactor at day 273. This increase in OLR was performed to evaluate whether the extended operating time could induce the anaerobic biomass in the digester to adapt to the substrate, so that the digester can operate at 1.5 g VS/(L·d). In the study carried out in **Chapter 5** it was observed a 54 % reduction in methane production at an OLR of 0.75 g VS/(L·d), with the same substrate pre-treated hydrothermally with steam explosion at 220 °C during 5 min.

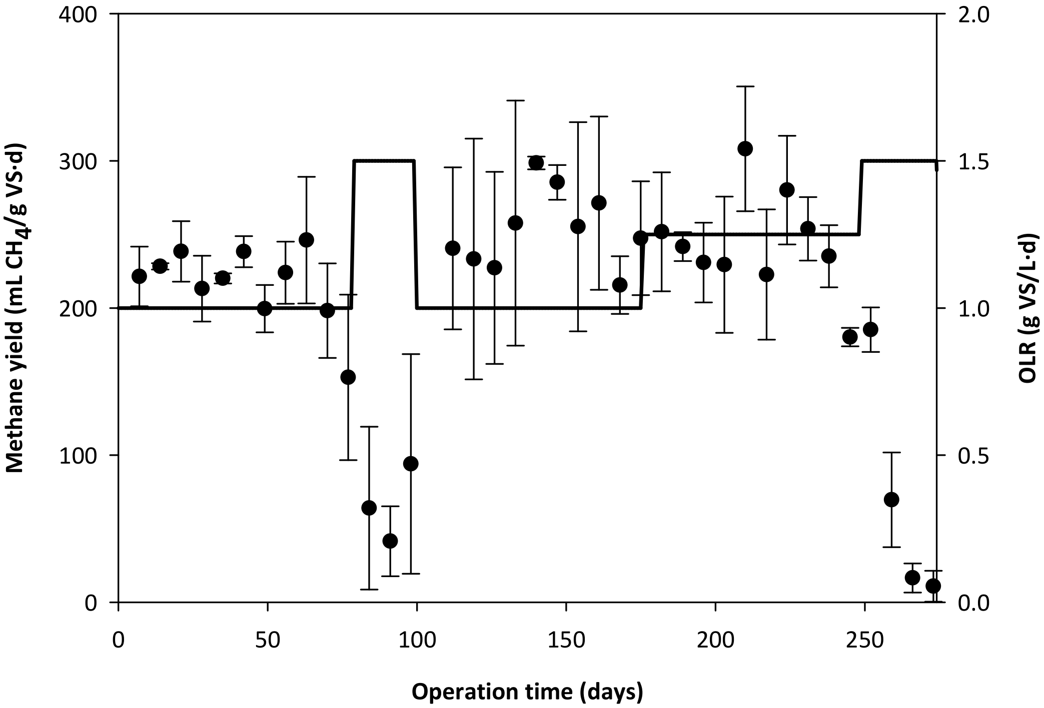

Figure 6.1. Variation of the methane production rates with their corresponding standard deviations and organic loading rates (OLR) during the whole experimental time.

Table 6.3. pH, alkalinity, TS, VS, sCOD, total VFA, total phenols, methane production rates and biodegradability during the different organic loading rates (OLRs).

	Stage 1	**Stage 2**	**Stage 3**	**Stage 4**	**Stage 5**
OLR (g VS/(L·d)	**1**	**1.5**	**1**	**1.25**	**1.5**
Days	0-77	78-98	99-174	175-247	248-273
pH	6.8±0.3	5.8±0.2	6.8±0.2	6.9±0.2	5.2±0.7
Alkalinity (mg $CaCO_3$/L)	1920±670	1200±500	2300±200	2200±600	900±400
TS (mg TS/L)	12693±2627	21145±3382	19046±3028	20346±3895	28699±1719
VS (mg VS/L)	10250±2875	18423±3015	16412±2844	17969±3920	26272±1521
sCOD (mg O_2/L)	891±432	5368±979	2780±1522	1296±188	6320±2748
Total VFA (mg O_2/L)	240±331	3842±1042	1313±1295	102±128	5068±3265
Ratio VFA/CODs (%)	27	72	47	8	80
Total phenols (mg gallic acid/L)	186±77	423±28	372±78	363±57	430±48
Methane production (mL CH_4/g VS d)	216±26	66±26	253±26	243±34	71±81
Biodegradability CH_4 (%)	27±5	12±3	30±9	32±4	2±1
Biodegradability VS (%)	70±2	61±8	67±15	69±9	58±7

6.3.2. Variation of organic matter soluble concentration and the stability of anaerobic process with operation time

In order to control the degradation of the organic matter fed and to monitoring the stability of the anaerobic digestion process with the variation in the OLR, the following parameters were daily measured: pH, alkalinity, sCOD, and VFA (**Figure 6.2 A and B**). In the first stage, i.e., 0–77 days and OLR = 1 g VS/(L·d), the sCOD presented an average value of around 891 ± 432 mg O_2/(L·d), being the ratio of VFA/sCOD of 27 %. There was also a decrease in pH and alkalinity values around of 6.4 and 1200 mg $CaCO_3$/L respectively. These decreases were also observed for the SE after a steam explosion, at 220 °C during 5 minutes, and phenol compounds recovery treatments and same OLR = 1 g VS/(L·d) in the study reported in **Chapter 5**. Therefore, with the change in OLR, sodium bicarbonate was added to the reactors to buffer the drop in pH with the aim to achieve values between 6.5-7.5, optimal range for an stable anaerobic process (Wheatley 1990; Mao et al. 2015). When the OLR was increased to 1.5 g VS/(L·d), between days 78-98, a marked increase in sCOD was observed, which presented an average value of 5368 ± 979 mg O_2/(L·d) with a ratio of VFA/sCOD of 72 %, related to inhibition in methane production due to the acidification of the reactors. The acidification of the reactors due to a high OLR for the anaerobic digestion of SE, caused an increase in the VFA due to an increase in the bacterial activity of the hydrolysis/acidogenesis stage, producing a decrease in pH (**Figure 6.2**) (Montañés et al. 2013). In addition, it also caused a simultaneous decrease in the microbial activity of the methanogenesis stage as was observed in the decrease in methane production (**Figure 6.1**) (Mao et al. 2015). In the stage 3, between days 99–174 days and operating again at an OLR = 1 g VS/(L·d) a stabilization of the reactors was observed after the inhibition process, achieving a decrease in the accumulation of sCOD, which presented a mean value of 2780 ± 1522 mg O_2/(L·d) and a ratio of VFA/sCOD of 47 %. Furthermore, stabilization of the reactors was also observed in the almost neutral pH values, i.e. 6.8 ± 0.2, and an alkalinity average value of 2300 ± 200 mg $CaCO_3$/L. With the increase in OLR to 1.25 g VS/(L·d), between days 175-247, the sCOD presented a stable mean value of around 1296 ± 188 mg O_2/(L·d) and a ratio of VFA/sCOD of 8 %. Moreover, pH was stabilized with a value of around 6.9± 0.2 and an alkalinity average value of 2200 ± 600 mg $CaCO_3$/L. However, when the OLR was again increased again to 1.5 g VS/(L·d), between days 248-273, a greater inhibition was observed in the methane production process, and an accumulation of sCOD was also found with an average value of around 6320 ± 2748 mg O_2/(L·d) and a ratio of VFA/sCOD of 80 %, completely inhibiting the reactor. During stage 5, alkalinity decreased until 900 ± 400 mg $CaCO_3$/L, causing an acidification of the reactors with average pH values of 5.2 ± 0.7 (**Table 6.3**). When the reactors were operated at an OLR of 1.5 g VS/(L·d) of OLR in stages 2 and 5 the alkalinity values were below than the ideal conditions for anaerobic digestion, achieving amounts between 2000 and 4000 mg $CaCO_3$/L (Fernández-Rodríguez et al. 2019; Casallas-Ojeda et al. 2021). These low alkalinity values may have been produced by a feed with pH values around 4 at high OLR (**Table 6.1**) that produced a pH drop in the reactor as described by Arhoun et al., (2017).

Individual VFAs, between C2-C5, were measured throughout the experiment and expressed in mg O_2/L (Figure 3). In stage 1, i.e., 0–77 days, and OLR = 1 g VS/(L·d), individual VFA concentrations were less than 50 mg O_2/L, except for propionic acid, for which its concentration increased to values of 350 mg O_2/L. In stage 2, i.e., 78–98 days, and OLR = 1.5 g VS/(L·d), a huge concentration of VFA was produced due to instability of the process, showing C2 and C3 mean values of around 1300 mg O_2/L, and i-C4, n-C4, i-C5 and n-C5 concentrations of less of 1000 mg O_2/L. At the end of stage 3, i.e., 99–174 days, the reactors were effectively recovered of the previous inhibition episode and the accumulation of VFA was solved, presenting mean values for each individual VFA at the end of the stage 3 below 50 mg O_2/L. Continuing this decreasing trend and remaining constant during stage 4 with OLR = 1.25 g VS/(L·d), i.e., 175–247. Finally, when the OLR was increased again up to 1.5 g VS/(L·d) in the stage 5, between days 248-273, a higher inhibition was observed, reaching concentrations greater than 1000 mg O_2/L for each acids studied. Acetate has been described as the least toxic fatty acid being propionate the most inhibitory acid at concentrations of approximately 3000 mg/L (Montañés et al. 2013). In another study it was described that acetic acid at concentrations higher than 800 mg/L can trigger a failure in anaerobic digestion process (Casallas-Ojeda et al. 2021).

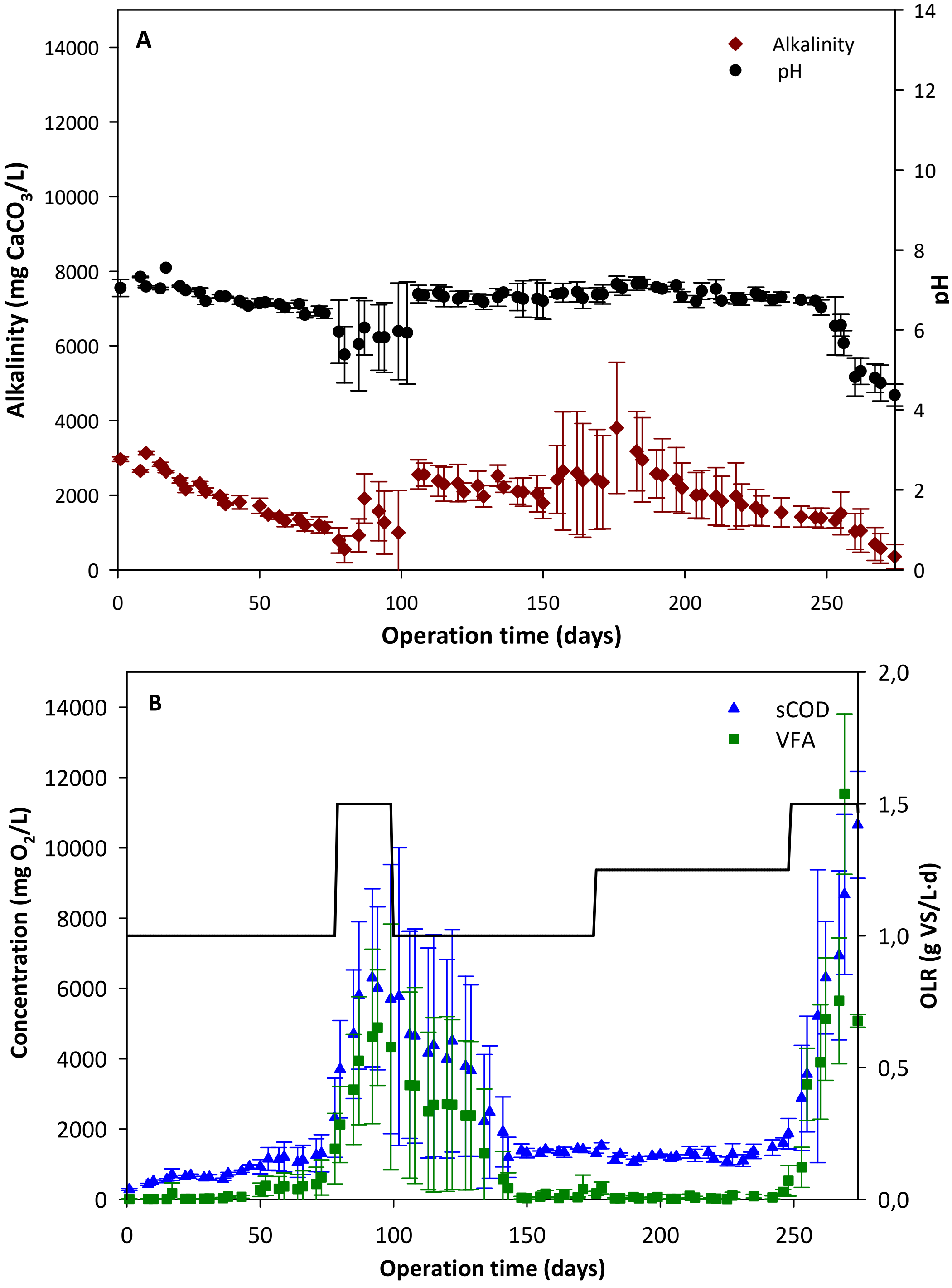

Figure 6.2. Variation of stability measured with (**A**) pH (●) and Alkalinity (◆) and Variation of organic matter measured with (**B**) sCOD (▲) and VFA (■) values with their standard deviations throughout the experimental period for the different organic loading rates (OLR) assayed.

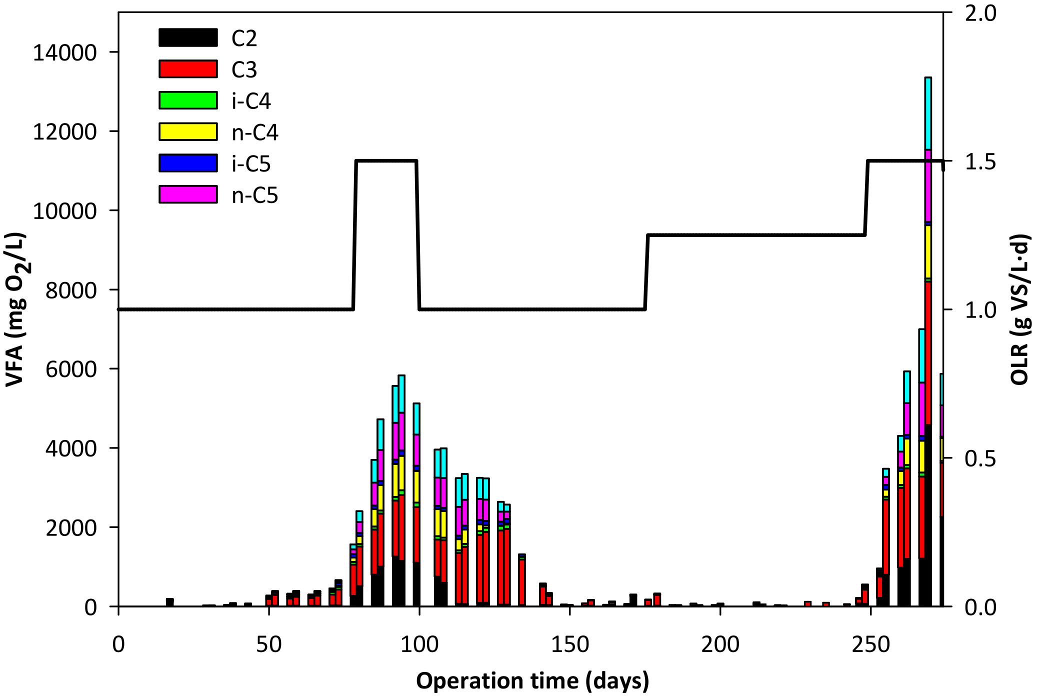

Figure 6.3. Variation of the VFAs values with their standard deviations throughout the different organic loading rates (OLR) during the experimental time.

6.3.3. Variation of the concentration of phenol compounds with organic loading rate throughout the experimental time

Phenol compounds, expressed as mg gallic acid/L, were determined because they are potential inhibitory compounds for the anaerobic digestion process (Figure 4) (Battista et al. 2015). During stages 1 and 2, despite of the OLR, the phenol compound concentration increased until reaching an average value of 423 ± 28 mg gallic acid/L. During stages 3 and 4 it is observed that there were no significant changes in the concentration of phenols having values around 350 mg gallic acid/L. When the reactors operated at unstable conditions due to the increase of OLRL at 1.5 g VS/(L·d) in stage 5, the phenol compound concentration achieved a value of 599 ± 64 mg gallic acid/L, (**Figure 4**). Microbial growth could be affected by a high concentration of phenol compounds (Borja et al. 1996; Battista et al. 2015; Caroca et al. 2021). However, the concentrations of phenols found in these tests are always lower than those considered to have an inhibitory effect on the anaerobic digestion process, i.e., 2000 mg/L (Calabrò et al. 2018). The increase in phenols was similar to that found in **Chapter 5** with the same substrate but treated with a steam explosion at 220 °C during 5 minutes. These concentrations of phenols were lower to those found in the study carried out with olive mill solid waste treated with the same process than that applied in this research (Serrano et al. 2019b).

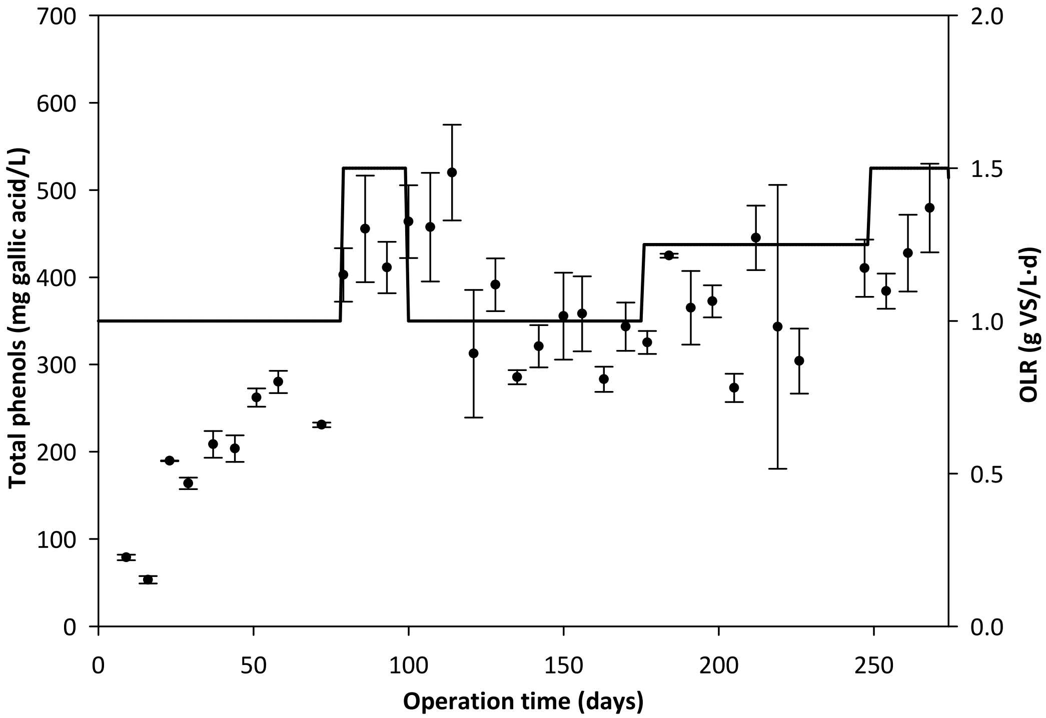

Figure 6.4. Variation of the total phenol concentrations with their standard deviation with the organic loading rate (OLR) throughout the experimental time.

6.3.4. Economic assessment

The minimum sales price of the phenol compound extract was calculated for a zero net profit, being EUR 0.812/g of gallic acid equivalents for economic viability at an OLR of 1.25 g of VS/(L·d) (**Figure 6.5**). In the study carried out in **Chapter 5**, in which the same strawberry extrudate was hydrothermally treated at 220 °C for 5 min, the price for phenol extract for zero net profit was EUR 0.610/g of gallic acid, but with a lower OLR of 0.5 g of VS/(L·d). The steam consumed in the hydrothermal treatment of SE at 170 °C of SE, 1.03 kg of steam/kg of SE, was much less than the values reported in the study of **Chapter 5**, i.e., 6.71 kg of steam/kg of SE. Moreover, the extracted phenol compounds were higher in the study of **Chapter 5**, i.e., 0.90 g of gallic acid/kg of extrudate from steam-explosion of SE at 220 °C versus 0.6 g of gallic acid/kg of SE in the present study with hydrothermal treated at 170 °C. However, in **Chapter 4**, a double concentration of phenols was extracted from the same SE with the same treatment conditions. According to these data, steam consumption was fully covered by the energy produced from the high methane production yield at an OLR of 1.25 g of VS/(L·d). In addition, the high recovery, despite of the low sales price, of the phenol compounds could make the economic assessment positive. However, another interesting option for the anaerobic digestion of SE after extraction of phenol compounds could be the mixing several residues to obtain a higher volume of organic matter to treat, and to design biomethane plants on a larger scale that is more economically sustainable (Baena-Moreno et al. 2020). Other management options could be to focus on the recovery of VFA, and/or soluble phenol compounds intead of biomethane during the anaerobic fermentation (Serrano et al. 2020). This option could be interesting to achieve an integral valorization of the SE at producing valuable building-block molecules useful for different industrial bioprocesses.

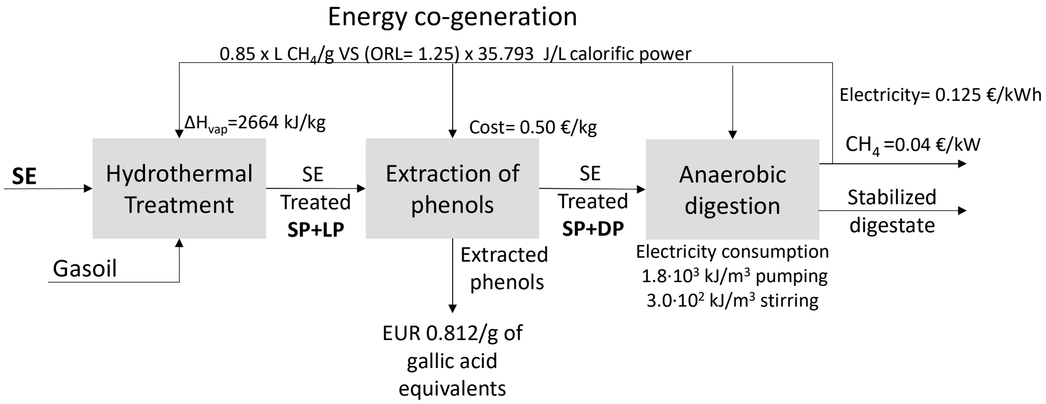

Figure 6.5. Block diagram of the valorization from SE based on **Chapter 3**.

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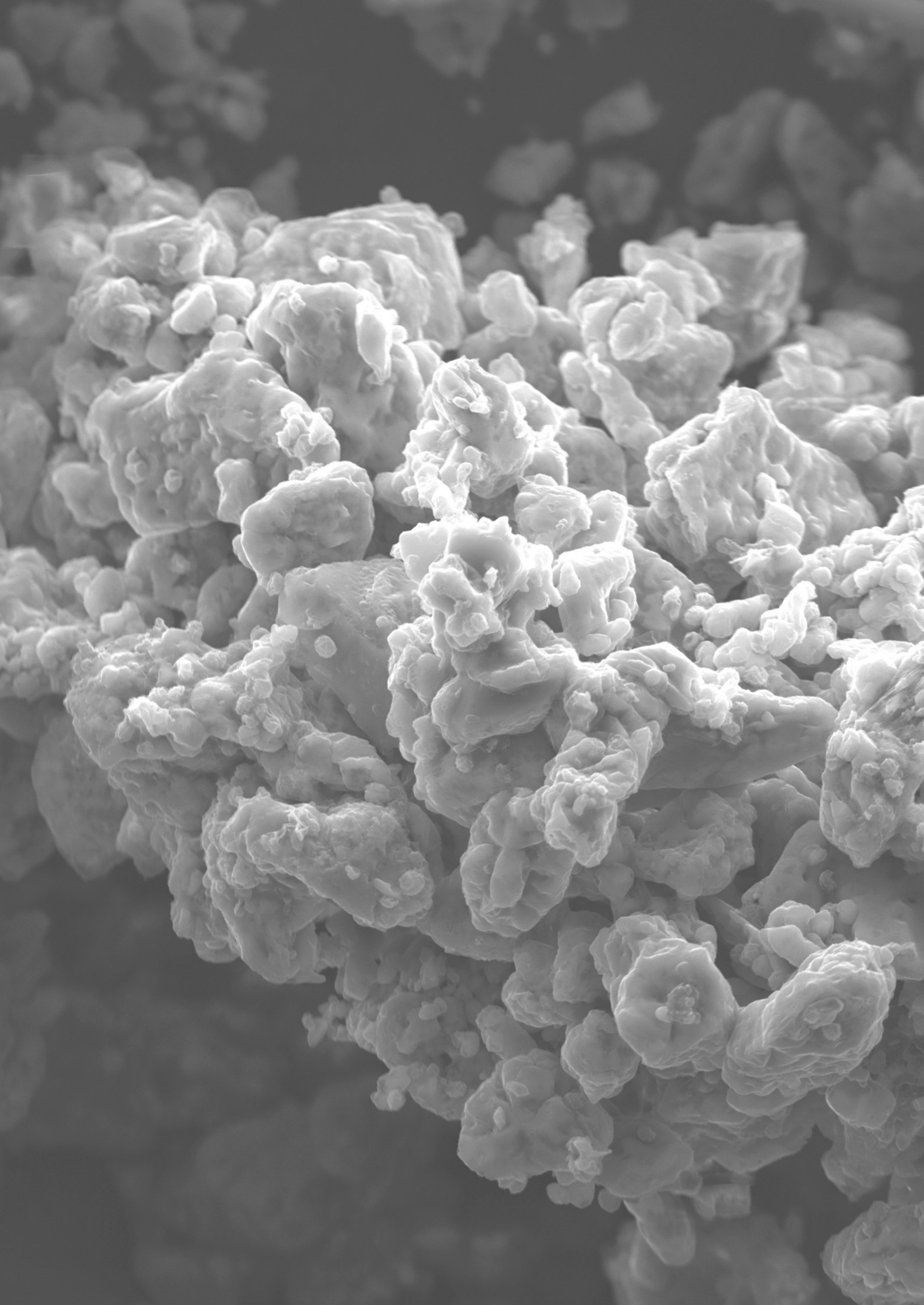


7

New antioxidant and antimicrobial materials based on sulfur by inverse vulcanization reaction

Abstract

The current great concern due to the contamination of plastics opens up trades for the production of more sustainable polymers. Inverse vulcanization has emerged as a novel reaction to achieve inorganic-organic hybrid polymeric materials. Sulfur is obtained after the oil refining process and makes inverse vulcanization a sustainable process due to the large amount of sulfur without a useful life. In previous studies, vegetable oils were used as a comonomer with sulfur to form copolymers. Nevertheless, compounds providing from agro-wastes such as phenol compounds from strawberry extrudate, could be a third comonomer that benefit subsequent copolymer. In this study, a series of copolymers with castor oil and 80 % of sulfur have been formulated by adding a third chemical compound with double bonds and heteroatoms. A study has been carried out to assess the antimicrobial capacity and antioxidant activity of the different copolymers obtained, to demonstrate the benefits of adding a new comonomer.

7.1. Introduction

Currently, there is great concern about the effect of plastics on the pollution of our planet, due to the exponential increase in its world production, which has gone from 1.5 Mt in 1950 to 335 Mt in 2016 (Geyer et al. 2017; Getor et al. 2020). Plastics play an important role in the packaging, agriculture, consumer electronics and motoring sectors, and only 1 % of the total annual production of plastics is bioplastics (Rujnić-Sokele and Pilipović 2017). Nowadays there is a large social movement that is committed to the production of bioplastics because the negative effects that occur such as their accumulation, global warming and the environmental pollution problems that conventional plastics cause in their life cycle (Bayer et al. 2014; Vilela et al. 2014; Heredia-Guerrero and Athanassiou 2016; Heredia-Guerrero et al. 2017). The preparation of more sustainable polymeric materials with novel structures and simultaneously interesting properties has a high priority in modern polymer chemistry (Wręczycki et al. 2020).

In a study by Chung et al., (2013) they developed the revolutionary inverse vulcanization concept that efficiently uses elemental sulfur (S_8) as a reaction medium and as comonomer with other compounds bearing double bonds, for the synthesis of new polymeric materials. Through inverse vulcanization could be obtained stable polymers with a very high sulfur content (50-90 % sulfur by mass) can be obtained. Currently, sulfur is the tenth most abundant element on Earth and it is valuable for the scientific community as chemical reagent with important uses in industry, agriculture or material science (Boyd 2016). Around 70 million tons of sulfur are obtained as a by-product each year, therefore, refined crude oil is currently the largest source of sulfur production (Apodaca 2014). Although several uses for sulfur have been previously described, there is still a net excess of sulfur that offer few economics uses, thus finding a new large-scale use for this sulfur would be an important development. Sulfur has a certain chemical inertness, exhibiting limited solubility in many organic solvents, except for poor solubility in some aromatics such as toluene, carbon disulfide and pyridine (Griebel et al. 2016). All of this makes it difficult to synthesize polymers directly from sulfur, under slight reaction conditions. The main limitations are overtaken by inverse vulcanization through ring-opening polymerization reaction (Griebel et al. 2016).The use of this new type of copolymers has opened a field to the study of new materials with very useful chemical and optical properties since they have uses in such important fields as materials science, polymer technology, adhesives, electrochemistry, or water treatment. Since it depends on the nature of the bond between the copolymers, the specific surface of the material formed can be modified, as well as its physical-chemistry properties (Gomez et al. 2016; Zhu et al. 2019; Wadi et al. 2020; Tikoalu et al. 2020).

The properties of the polymers could be controlled by the selection of the polymerization reaction conditions and the use of appropriate comonomer, obtaining a wide variation of properties depending on the reagents used. Vegetable oils can be considered an effective option for its use as a green comonomer. Taking advantage of their properties, they can range from highly cross-linked thermosets to linear thermoplastics, making them applicable in a wide range of materials for various uses (Adekunle 2015; Abbasi et al. 2019). Recently, some system have been described that take advantage of some properties of vegetable oils, elaborating new polymeric materials with natural origin from vegetable oils as raw material, obtaining materials for many applications (Abbasi et al. 2019). Based on these precedents, other copolymers could also be formed from natural origin such us, strawberry extrudate, which is a source of a great variety of bioactive compounds, as mentioned in **Chaper 1**. These bioactive compounds can be used as comonomers for the inverse vulcanization process. A very recent example of the use of an agro-waste, as a source of a comonomer compound, such as limonene has been reported in the literature for use as a comonomer in the inverse vulcanization process (Crockett et al. 2016). The use of copolymers of natural origin that come from residues of the agro-food industry would lead to obtaining polymeric material that offers a high degree of biodegradability, antioxidant power, and antimicrobial properties, and can be found in the examples recently described in the literature (Griebel et al. 2016; Smith et al. 2020; Guo et al. 2021).

A green option to obtain copolymers with sulfur by inverse vulcanization have been carried out with vegetable oils, due to the easy interaction with the unsaturations of the triglycerides. We can found that sunflower, linseed, and olive oils were applied in the development of lithium-sulfur battery cathodes (Gomez et al. 2016; Hoefling et al. 2017). In another study, soybean oil was chosen to react with elemental sulfur to be investigated as a source of sulfur for plants, as a fertilizer, with improved oxidation capacity (Valle et al. 2019). Sulfur-based polymers obtained from the inverse vulcanization of sulfur with canola oil and recycled cooking oils to produce an adsorbent for mercury capture were also investigated.

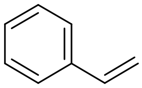
Copolymers formed in previous studies with good properties could be improved by adding a new chemical compound with specific properties for industry. A new generation of polymers is intended to have antioxidant and antimicrobial properties for the formation of new plastics with these interesting properties. Phenol compounds have been reported as biactive compounds and with antioxidant and antimicrobial properties (Petkova et al. 2022) (**Chapter 1**). Compounds such as cinnamic acid from strawberry or bisphenol A can be an example of the formation of copolymers with bioactive compounds from this fruit. In addition, bisphenol A is a compound that is found in fruit packaging and is causing harm to human health (Sun et al. 2020; Khan et al. 2021). Furthermore, the incorporation of heteroatoms (oxygen and nitrogen) in the main chain of the copolymer with these new chemical compounds can favor different properties such as antimicrobial and antioxidant properties (Chatzimitakos et al. 2020; Das et al. 2021). Based on this premise, other compounds that can infer interesting characteristic to the final polymer material could be imidazole, one of the most used catalysts in chemical synthesis, that was also been proposed to increase the reaction rate in this kind of copolymerization (Moraes et al. 2017). Other compounds that carry double bonds and are suitable to have biological application is styrene, which was previously used as a comonomer in another study to obtain electric devices (Lin et al. 2019; Wang and Zhang 2020; Wręczycki et al. 2020). Likewise, another candidate which could also favour the kinetics of the reaction is the triallylamine (Yasin et al. 2020; Tonkin et al. 2020).

The aim of this work is the generation of new kind of copolymers by inverse vulcanization with S_8, and natural products for the formation of antioxidant and antimicrobial polymeric materials.

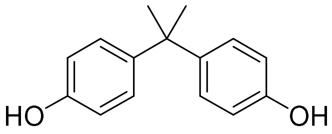
7.2. Materials and methods

7.2.1. Raw materials

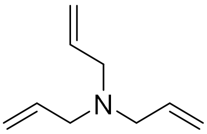
Castor Oil (CO) was supplied by Ginama (Valencia, Spain) and elemental Sulfur (S_8) 99 % was supplied by the refining petroleum company CEPSA (Huelva, Spain). Third compounds were Styrene (STY), Biphenol A (BPH), Triallylamine (TAA), Imidazole (IMI) (Sigma-Aldrich, Steinheim, Germany) and Cinnamic acid (CIN) (Acros Organics, Geel, Belgium) (**Figure 7.1**).



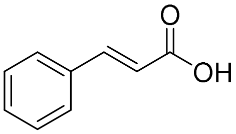
Styrene (STY)



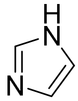
Bisphenol A (BPA)



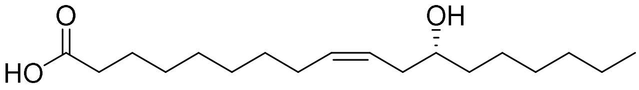
Triallylamine (TAA)



Cinnamic acid (CIN)



Imidazole (IMI)



Castor oil (CO)

Figure 7.1. Different comonomers used for the generation of copolymers.

7.2.2 Synthesis of new materials by inverse vulcanization

Inverse vulcanization of elemental Sulfur, castor oil (CO) and a third compound were prepared following a slight modification of Chalker's preparation (Tikoalu et al., 2020). S_8 and CO were heated and melted under vigorous stirring until 170 °C within an oil bath in a round bottom flask for 60 min with S/CO:80/20 ratio (**Figure 7.2**). For ternary copolymers when blends S_8 and CO were heated at 170 °C, the third compound was slowly added. Blends were stirred for 60 min keeping the temperature in order to finish the comonomers conversion. It was made 20 g of each new following copolymers: S/CO/STY:80/14/6, S/CO/BPA:80/14/6, S/CO/TAA:80/14/6, S/CO/CIN:80/14/6 and S/CO/IMI:80/14/6.

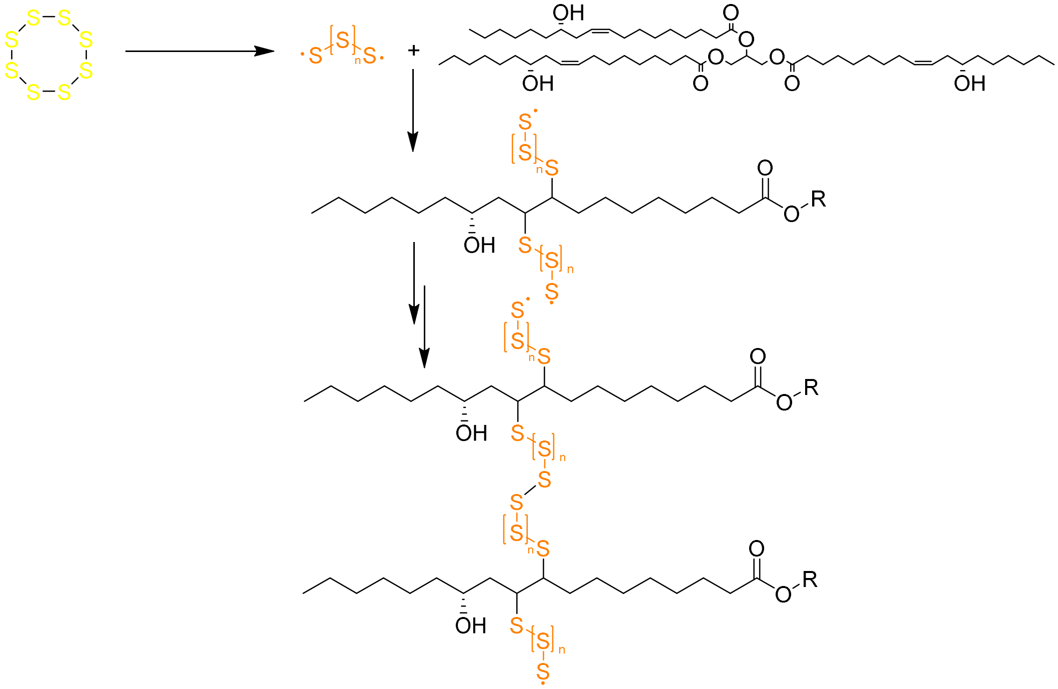


Figure 7.2. Sulfur and castor oil synthesis process by inverse vulcanization.

Figure 7.3. (S/CO/X) copolymers generated with different chemical compounds.

In addition, a new strategy to obtain copolymers to improve their future biological capacities is based on the elimination of vegetable oil as comonomer, and the use of best possible activity compounds as the second comonomer. Based on the previous chapters oh this thesis, polyphenols would be perfect candidates. For this, the introduction of double bonds in its structure is necessary., in our study was tested bisphenol A as a comonomer, that previously had suffered an allylation This procedure is based on a literature precedent (Karunarathna et al. 2019). In the flask was added 20 g of BPA with 800 mL dissolution of 0.5 M NaOH(aq)/acetone (1:4). Allyl bromide 20 mL was added and the mixture was allowed to heat for 5 hours at 40 °C with continuous stirring. After 5 hours, the solution was concentrated on leaving it in the extractor hood overnight. The remaining solution was acidified to pH 2 by dropwise addition of concentrated HCl(aq), resulting was introduced in a separating funnel and washed four time with distilled water and another with hexane. Finally, it was left to dry in a hood for 48 hours and the polymer was formed by inverse vulcanization with an S/BPA-allyl:80/20 ratio as previously described (**Figure 7.4**).

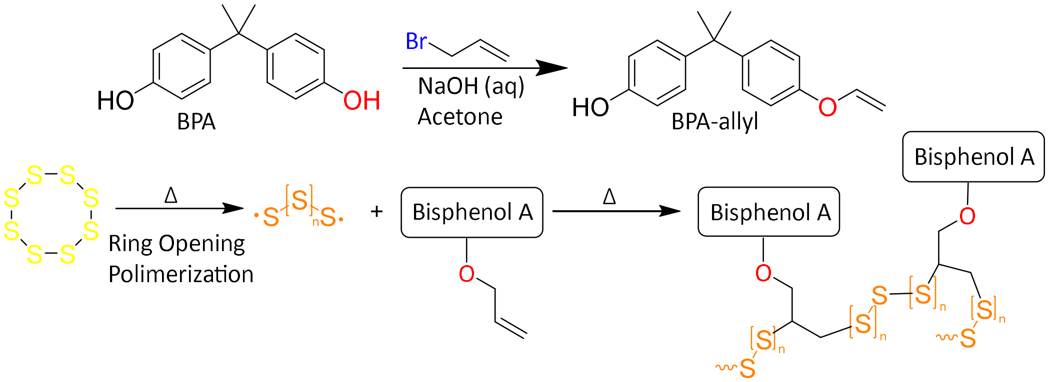


Figure 7.4. Allylation reaction of bisphenol A with allyl bromide, and obtaining of polymer by inverse vulcanization.

7.2.3. Antioxidant activity assay

The dissolution of the copolymers in different organic solvents was tested with pyridine, dichloromethane, acetone, dimethyl sulfoxide, carbon disulfide and Tetrahydrofuran, obtaining it only in carbon disulfide and pyridine. 0.1 g of each material was dissolved in 25 ml of disulfide carbon to measure the antioxidant power. The antioxidant activity of the new material obtained was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) spectrophotometric method (Brand-Williams et al. 1995), and was expressed as milligrams of Trolox equivalents per g polymer (mg TEAC/g polymer).

7.2.4. Antimicrobial activity assay

The antimicrobial activity of the polymers was tested against two bacterial strains pathogenic to humans, *Escherichia coli* (Gram −) and *Bacillus cereus* (Gram +), and the yeast *Saccharomyces cerevisiae*. These microorganisms were grown in test tubes in an orbital shaker at 100 rpm until the cultures reached the stationary phase. Bacteria were cultured in LB medium at 37°C, while yeasts were grown in YPD medium at 30°C. An inoculum of 100 µL was then added to 4 mL of culture medium containing 100 mg mL^{-1} of each copolymer. A test tube without any polymer was used as a control of the microbial growth, Castor oil and S_8 were used as controls of the specific activity of the copolymers. The microorganisms were cultured overnight in the presence of the different copolymers and the inhibition of growth was measured through the optical density of the cultures at 600 nm in a spectrophotometer (UltrospectTM 3100 pro, LabWrench) in comparison with the control culture without copolymers.

7.2.5. Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance spectroscopy (^1H NMR) was used to determine the chemical structures of the copolymers. ^1H NMR of copolymers samples were recorded at 500 MHz using a Varian Mercury 500 spectrometer. Deuterated pyridine-d5 was used as solvent (δ= 7.18, 7.55, 7.70 ppm). ^1H NMR shifts are reported relative to tetramethylsilane.

7.3. Results and discussion

7.3.1. Structural analysis of the new copolymers via solution NMR Spectroscopy

Nuclear magnetic resonance spectroscopy was used to determine the chemical structures of the castor oil and the other copolymers synthesized. The representative ^1H NMR spectra, taken by dissolving the samples in deuterated pyridine-d5, of castor oil and new copolymers are shown in **Figure 7.5**. As can be clearly observed in the **Figure 7.5**, the alkene = CH proton peaks at δ = 5.60 and δ = 5.80 ppm present in castor oil are completely absent or overhead by peaks for other copolymers. In the study realized by Tikoalu et al.,(2020) similar behavior was obtained in the inverse vulcanization process with castor oil and sulfur. Regarding the new C-S bonds that should appear at δ = 1.30 ppm in the 1H NMR spectra but are masked, previous studies showed that sulfur-based copolymers should present signals due to the thiol group around 1.5 ppm (Wadi et al. 2018). The HOD peak appears around δ =5 ppm belonging to the impurities of deuterated water (Fulmer et al. 2010).

Different peaks appear belonging to the aromatic region from new copolymers against castor oil and S/CO:80/20 copolymer (**Figure 7.5**). Imidazole peaks appear in δ =7.03, 7.55 and 8.16 ppm from the S/CO/IMI copolymer similar to that described in other reaction by Butt et al., (2020). Cinnamic acid double peaks appear in δ = 6.91, 7.33, 7.60 and 8.03 ppm from the S/CO/CIM copolymer. Similar chemical shift of cinnamic acid was described in the study by Bezerra França et al., (2021). For the S/CO/TAA copolymer it does not appear any peaks representative from triallylamine, because it is masked with castor oil. Peaks from bisphenol A appear in δ =7.12 and 7.27 ppm in the S/CO/BPA copolymer similar to described in other reaction by van den Hoek and Fossum (2017). Finally, in the aromatic region from styrene multiple peaks appear around δ =7.29 ppm from S/CO/STY. Wręczycki et al., (2020) described similar aromatic peaks suggesting that the phenyl groups are located randomly along the macromolecules since the two peaks of the aromatic region which were observed in other copolymers did not appear.

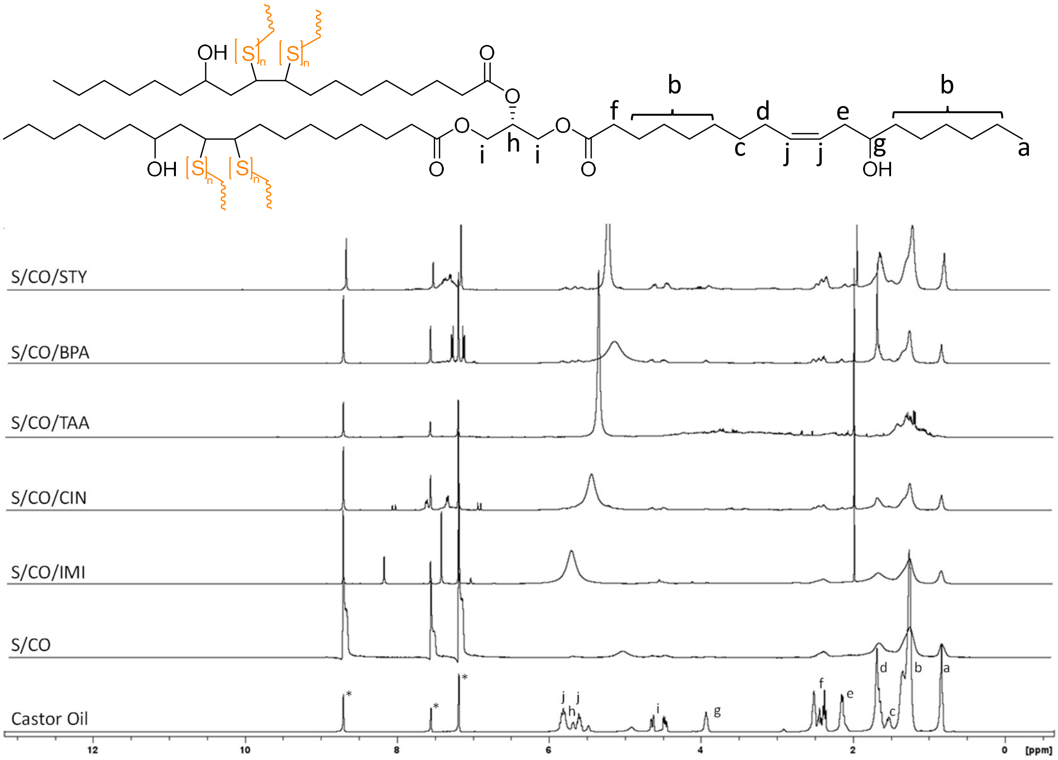


Figure 7.5. Castor oil structure and ^{1}H NMR of each copolymer and castor oil.

7.3.2. Study of antioxidant activity from the new copolymers

The antioxidant capacity of the new copolymers was calculated by means of DPPH radical scavenging methods and compared with the antioxidant activity of castor oil and sulfur. As can be seen in **Figure 7.6**, the S_8 has a very low radical scavenging capacity respect to the other sustances meassured. In a study as inorganic sulfur salts, Na_2S and Na_2S_2 were used to observe if they had antioxidant activity and no reduction of DPPH was observed similar to observed with S_8 (Xu et al. 2020). On the contrary, castor oil has been observed that have antioxidant activity and increases over time. This antioxidant activity of castor oil is derived from the phenol compounds of the oil itself (Park et al. 2020). In the copolymer S / CO has observed a little increase of antioxidant activity with respect to castor oil. Therefore, the addition of another compound that has antioxidant capacity in the copolymer could increase this property.

Copolymers with STY, IMI, and CIM have increased their antioxidant activity front to copolymer with only CO. However, copolymers with BPA and TAA have decreased with respect to copolymer S/CO. A higher antioxidant activity is observed when the copolymers have hydrophobic compounds such as styrene or imidazole (Latos-Brozio et al. 2021). The hydroxyl groups of S/CO/BPA responsible for scavenging DPPH radicals could be connected to the network during polymerization, as a result of which their ability to scavenge DPPH radicals decreased (Latos-Brozio et al. 2021). Furthermore, the hydroxyl groups of bisphenol A were not detected at the ^1H NMR of the S/CO/BPA copolymer in **Figure 7.6**. New polymeric materials from agro-wastes with high antioxidant capacity and moderate mechanical properties are being an alternative to replace traditional plastic containers (Santos et al. 2021). Therefore, this new generation of copolymers synthesized with sulfur and compounds from agro-wastes could be very promising.

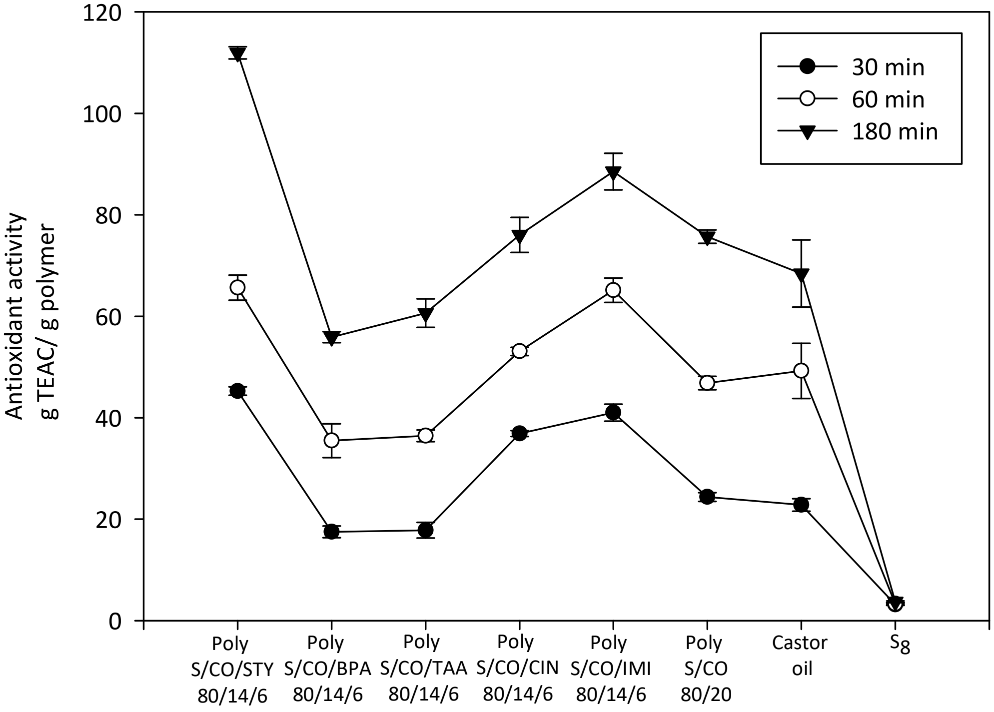


Figure 7.6. Antioxidant activities from different new copolymers determined by DPPH.

7.3.3. Antimicrobial properties

In the current situation of emergence of multi-factor resistant microbes, it is necessary to look for various materials resistant to bacteria and yeast from natural sources. In the present study the antimicrobial activity of new copolymers were assessed against two bacterial Gram - (*E. coli*), Gram + (*B. cereus*) and a yeast (*S. cerevisiae*). According to growth inhibitions, the results reflect that the extracts showed relatively good inhibitory activity against the tested bacteria (**Figure 7.7**). In the case of *E. coli*, growth inhibition was shown by castor oil being 44 % inhibitory. In a recent study it was shown that the hydrophobic ricinoleic acid chains of CO could pierce the bacteria attached to the copolymers, forming holes that cause leakage, lysis and death of the cytoplasm (Gharibi et al. 2021).

The new copolymers had an inhibition similar to castor oil against *E.coli*, the most significant case being BPA with 49 % (**Figure 7.7**). In the case of B. cereus, a significant effect was shown with the different new copolymers. It is observed that sulfur and castor oil had no significant effect on the growth of Gram + bacteria. Phenol compounds such as BPA and CIM had a great effect on the growth of *B. cereus*, inhibiting its growth by around 90 % (**Figure 7.7**). Furthermore, it was observed that IMI inhibited growth by 93 % and that only 61 % was inhibited with TAA. Finally, STY was only 34 % inhibited and the

S / CO polymer had no significant effects on growth (**Figure 7.7**). The hydrophobic chains of the copolymers might have less affected the growth of Gram - over Gram + bacteria due to the more hydrophobic phospholipid composition of the cell wall of Gram – bacteria (Gharibi et al. 2021). Furthermore, lipoproteins and phospholipids are found on the surface of Gram - bacteria, whereas the surface of Gram + bacteria contains abundant pores and has a much higher negative surface charge (Wang et al. 2017; Omerović et al. 2021). In the case of *S. cerevisae*, an inhibition by S_8 of around 63 % was observed, presenting a great inhibition in yeasts as observed in most of the new copolymers. CIM is a new, more inhibitory copolymer against *S. cerevisae* with a growth inhibition of 76 % (**Figure 7.7**). The lack of a great antimicrobial effect may be due to the low solubility of the copolymers in water and, therefore, to the difficulty of penetrating the cells (Latos-Brozio et al. 2021).

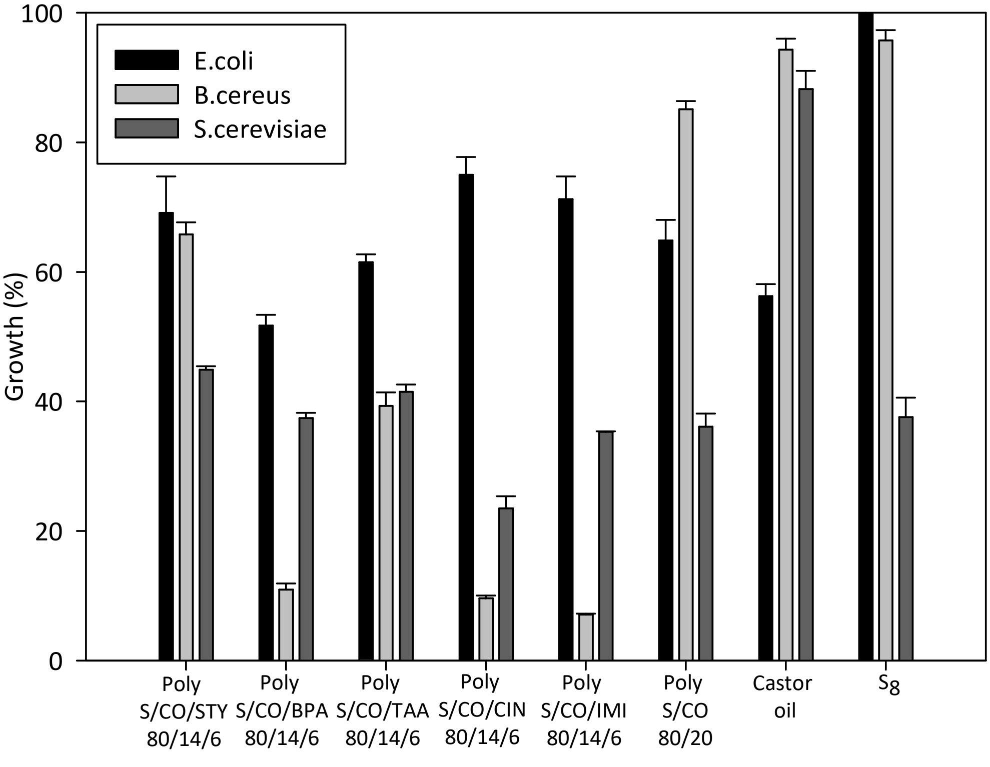


Figure 7.7. Growth inhibition assay of Gram - and Gram + bacteria and yeast against new copolymers.

7.3.4. Promising strategy for a new generation of polymers with sulfur and phenol compounds

A new study for the use of the waste product such as lignin has used the inverse vulcanization process to form new copolymers (Karunarathna et al. 2019). Starting from the substitution of the hydroxyl groups of the lignins with allyl bromide, it can be favored the reaction of allyl lignin with S_8, whereby part of the sulfur forms cross-links of polysulfide with lignin to produce a three-dimensional network. This study gives new avenues of studies with compounds with hydroxyl groups for the formation of new copolymers from the formation of allylic compounds and S_8 by inverse vulcanization. **Figure 7.8** shows the process of formation of new copolymers that could have a greater antioxidant and antimicrobial capacity from the strawberry phytochemicals shown in **Chapter 1**.

A previous study with BPA has been carried out for the formation of the allylation of the hydroxyl of BPA (BPA-Allyl) and subsequently, the inverse vulcanization reaction has been carried out. The formation of the two synthesized compounds (S/BPA-allyl:80/20) has been characterized by ^1H NMR. The reaction of BPA with allyl bromide (BPA-allyl) is observed to have been carried out almost completely since no signal is observed in $\delta = 11.30$ ppm of the hydroxyl group with respect to the unreacted BPA (**Figure 7.9**). In addition, it is observed that the signals of the double bond of the allyl group appear in $\delta = 5.20$, 5.44, and 6.06 ppm. Finally, when BPA-allyl reacts by inverse vulcanization with sulfur (S/BPA-allyl), the peaks of the double bond belonging to the allyl group disappear, due to the union with sulfur (**Figure 7.9**). These facts imply that this new strategy is valid for continuing research with phenols obtained from strawberry extrusion, being able to offer new properties and applications to future polymers.

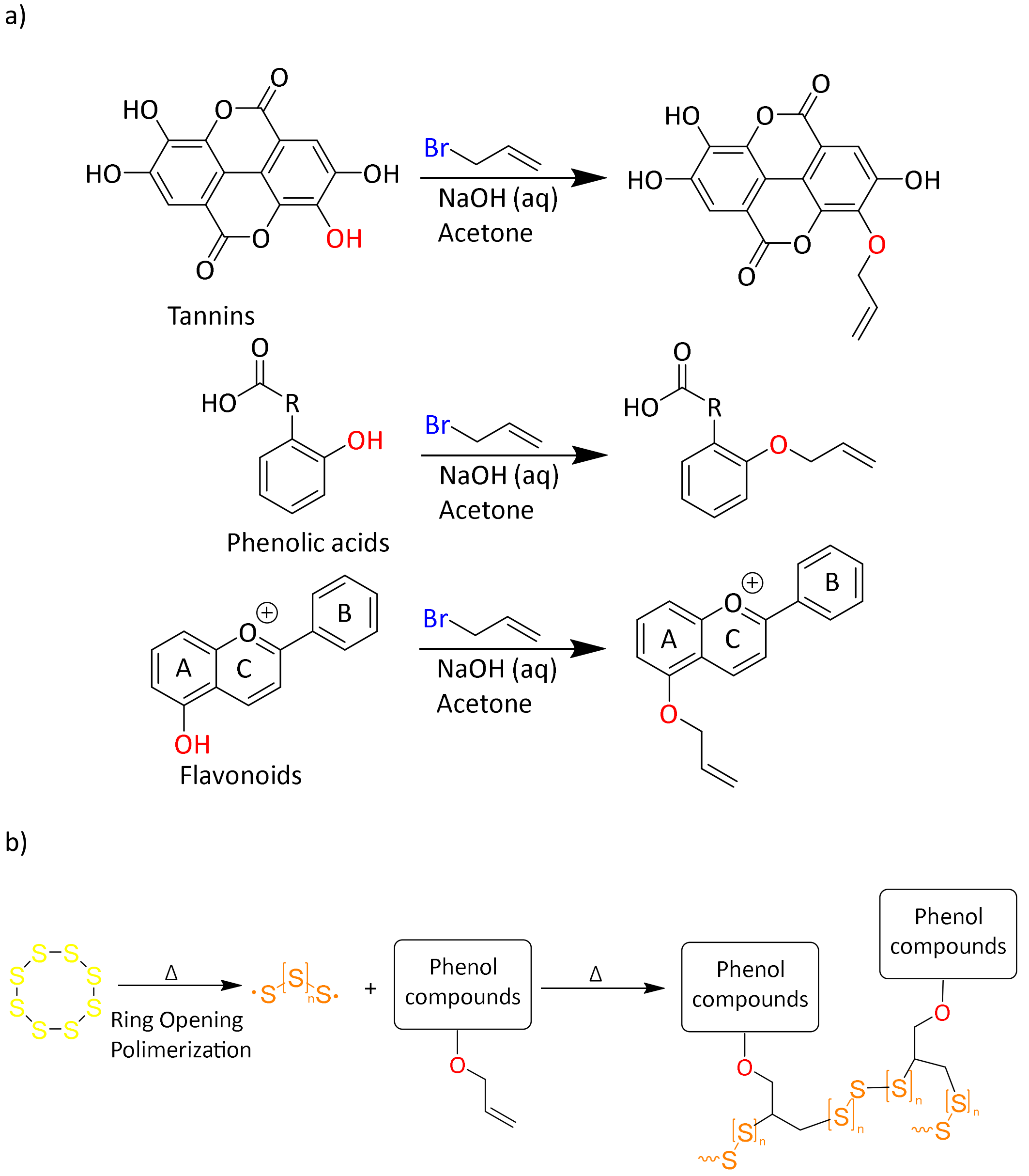


Figure 7.8. a) synthesis of substitution of the hydroxyl groups of the phytochemicals from strawberry extrudate with allyl bromide, b) synthesis of new polymeric materials obtained from phenol compounds from strawberry by inverse vulcanization

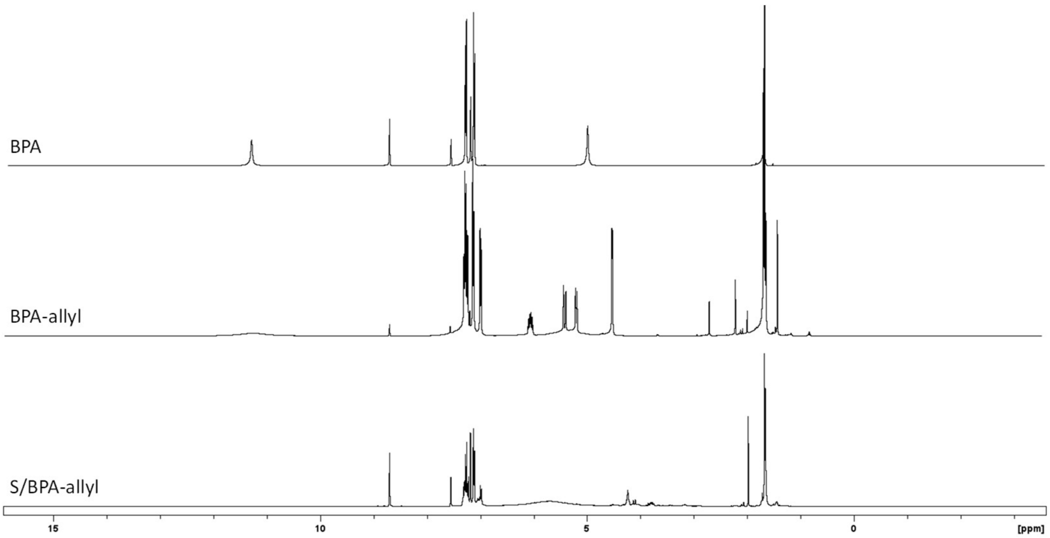


Figure 7.9. ^{1}H NMR of BPA and BPA-allyl from the reaction with allyl bromide, and ^{1}H NMR of BPA-allyl obtaining by inverse vulcanization.

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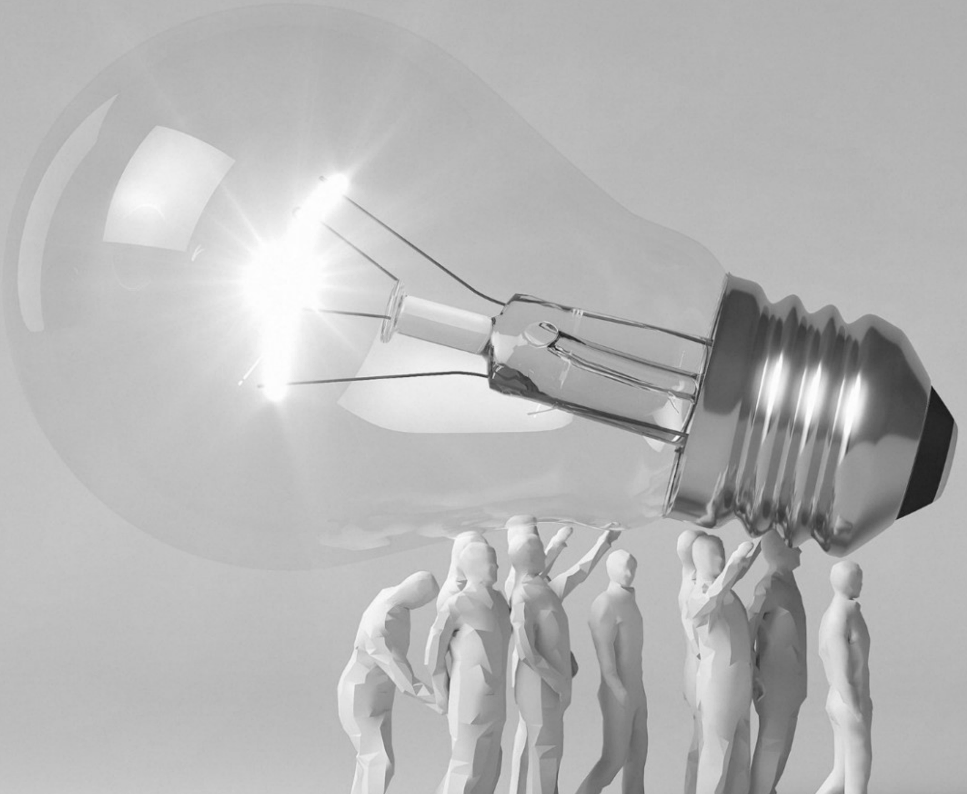

8 Conclusions

The results obtained in this PhD Thesis reveal the importance of the adequate management of agro-wastes, as well as the need of developing and improving some technologies for a good and suitable valorization of the strawberry extrudate.

- After extensive bibliographic review It has demonstrated that a promised option to solubilize bioactive compounds from strawberry extrudate is the hydrothermal treatment, in which a liquid fraction is obtained containing these compounds that can be extracted by means of an adsorbent resin. Bioactive compounds obtained, could be used as comonomers in the production of copolymers by inverse vulcanization. Finally, an anaerobic digestion process could be performed for stabilizing and obtaining methane from strawberry extrudate after extraction of the bioactive compounds to achieve a complete biorefinery process from strawberry extrudate (**Chapter 1**).
- Hydrothermal pre-treatments from strawberry extrudate have been studied in a range from 90 °C to 200 °C with and without acetic acid addition to favor the solubilization of organic matter. The optimal hydrothermal pre-treatment for obtaining high added value compounds, such as sugars and phenol compounds, was found at 150 °C without acetic acid for 60 minutes (**Chapter 2**).
- Three studies of valorisation with the same strawberry extrudate were proposed by using hydrothermal treatment at 150 °C, 170 °C, and 220 °C, adsorption of phenol compounds with Amberlite XAD 16 resin and a study of the anaerobic digestion of the remaining biomass (SP+DLP) in batch mode through biochemical methane potential tests. The hydrothermal pre-treatments at 170 °C and 220 °C have been the most optimal in the valorization of the strawberry extrudate by simultaneously obtaining phenol compounds and methane production (**Chapter 3 and 4**).
- Hydrothermal pre-treatments at 170 °C and 220 °C have been chosen to extend the study of anaerobic digestion in a semi-continuous regime at different organic loading rate. The steam explosion pre-treatment at 220 °C was more effective for the solubilization and recovery of phenol compounds, but the highest treatment capacity and methane production was achieved for the hydrothermal treatment at 170 °C. Therefore, the results showed that the treatment at 170 °C can be considered as the most adequate for the integral valorization of the strawberry extrudate (**Chapter 5 and 6**).
- Finally, different copolymers were made by inverse vulcanization with sulfur, castor oil and a third chemical compound, one of which was cinnamic acid, a bioactive compound from the strawberry extrudate. Depending on bioactive compounds used, new properties in the copolymers, such as antioxidant and

antimicrobial properties could be implemented. Therefore, this method of obtaining new copolymers by inverse vulcanization is an interesting option for the use of the bioactive compounds from the strawberry extrudate (**Chapter 7**).

Los resultados obtenidos en esta Tesis Doctoral revelan la importancia del manejo adecuado de los agro-residuos, así como la necesidad de desarrollar y mejorar algunas tecnologías para una buena y adecuada valorización del extrusionado de fresa.

- Tras una extensa revisión bibliográfica se ha demostrado que una opción prometida para solubilizar compuestos bioactivos del extrusionado de fresa es el tratamiento hidrotermal, en el que se obtiene una fracción líquida que contiene estos compuestos que se pueden extraer mediante una resina adsorbente. Los compuestos bioactivos obtenidos, podrían usarse como comonómeros en la producción de copolímeros por vulcanización inversa. Finalmente, se podría realizar un proceso de digestión anaeróbica para estabilizar y obtener metano a partir del extruido de fresa después de la extracción de los compuestos bioactivos para lograr un proceso de biorrefinería completo a partir del extrusionado de fresa (**Capítulo 1**).
- Se han estudiado pretratamientos hidrotermales a partir de extrusionado de fresa en un rango de 90 ° C a 200 ° C con y sin adición de ácido acético para favorecer la solubilización de la materia orgánica. El pretratamiento hidrotermal óptimo para la obtención de compuestos de alto valor agregado, como azúcares y compuestos fenólicos, se encontró a 150 ° C sin ácido acético durante 60 minutos (**Capítulo 2**).
- Se propusieron tres estudios de valorización con el mismo extrusionado de fresa mediante tratamiento hidrotermal a 150 ° C, 170 ° C y 220 ° C, adsorción de compuestos fenólicos con resina Amberlite XAD 16 y un estudio de digestión anaeróbica de la biomasa remanente (SP + DLP) en modo por lotes mediante pruebas de potencial bioquímico de metano. Los pretratamientos hidrotermales a 170 ° C y 220 ° C han sido los más óptimos en la valorización del extruido de fresa al obtener simultáneamente compuestos fenólicos y producción de metano (**Capítulos 3 y 4**).
- Se han elegido pretratamientos hidrotermales a 170 ° C y 220 ° C para ampliar el estudio de la digestión anaeróbica en un régimen semicontinuo a diferentes tasas de carga orgánica. El pretratamiento por explosión de vapor a 220 ° C fue más efectivo para la solubilización y recuperación de compuestos fenólicos, pero la mayor capacidad de tratamiento y producción de metano se logró para el tratamiento hidrotermal a 170 ° C. Por tanto, los resultados mostraron que el tratamiento a 170 ° C puede considerarse como el más adecuado para la valorización integral del extrusionado de fresa (**Capítulos 5 y 6**).
- Finalmente, se elaboraron diferentes copolímeros mediante vulcanización inversa con azufre, aceite de ricino y un tercer compuesto químico, uno de los cuales fue el ácido cinámico, un compuesto bioactivo del extrusionado de fresa. Dependiendo de los compuestos bioactivos utilizados, se podrían implementar nuevas propiedades en los copolímeros, como propiedades antioxidantes y

antimicrobianas. Por tanto, este método de obtención de nuevos copolímeros por vulcanización inversa es una opción interesante para el uso de los compuestos bioactivos del extruido de fresa (**Capítulo 7**).

List of scientific production

1. **Cubero-Cardoso, J**., Serrano, A., Trujillo-Reyes, Á., Villa-Gómez, K.D., Borja, R., and G. Fermoso, F. 2020. Chapter title: Valorization options of strawberry extrudate agro-waste. A review. Book title: *innovation in the food sector through the valorization of food and agro-food by-products*. IntechOpen. doi:10.5772/intechopen.93997. A slightly modified version is presented in **Chapter 1**.

2. Rodríguez-Gutiérrez, G., **Cubero Cardoso, J**., Rubio-Senent, F., Serrano, A., Borja, R., Fernández-Bolaños, J., and Fermoso, F.G. 2019. Thermally-treated strawberry extrudate: A rich source of antioxidant phenols and sugars. *Innovative food science and emerging Technologies*. 51: 186–193. Elsevier. doi:10.1016/j.ifset.2018.05.017. A slightly modified version is presented in **Chapter 2**. Impact factor (2019): 4.477.

3. Trujillo-Reyes, Á., **Cubero-Cardoso, J**., Rodríguez-Gutiérrez, G., García-Martín, J.F., Rodríguez-Galán, M., Borja, R., Serrano, A., and Fermoso, F.G. 2019. Extraction of phenolic compounds and production of biomethane from strawberry and raspberry extrudates. *Biochemical engineering journal*. 147: 11–19. Elsevier. doi:10.1016/J.BEJ.2019.03.023. A slightly modified version is presented in **Chapter 3**. Impact factor (2019): 3.475

4. **Cubero-Cardoso, J**., Trujillo-Reyes, Á., Serrano, A., Rodríguez-Gutiérrez, G., Borja, R., and Fermoso, F.G. 2020. High-value-added compound recovery with high-temperature hydrothermal treatment and steam explosion, and subsequent biomethanization of residual strawberry extrudate. *Foods* 9(8): 1082. MDPI. doi:10.3390/foods9081082. A slightly modified version is presented in **Chapter 4**. Impact factor (2020): 4.350.

5. **Cubero-Cardoso, J**., Muñoz-Arjona, A., Trujillo-Reyes, Á., Serrano, A., Alonso-Fariñas, B., Rodríguez-Gutiérrez, G., Urbano, J., Borja, R., and Fermoso, F.G. 2020. Mesophilic semi-continuous anaerobic digestion of strawberry extrudate pretreated with steam explosion. *Foods* 9(12): 1887. MDPI. doi:10.3390/foods9121887. A slightly modified version is presented in **Chapter 5**. Impact factor (2020): 4.350.

6. **Cubero-Cardoso, J**., Jimenez-Páez, E., Trujillo-Reyes, Á., Serrano, A., Rodríguez-Gutiérrez, G., Borja, R., Urbano, J., and Fermoso, F.G. Valorization of strawberry extrudate waste: recovery of phenol compounds by high-temperature hydrothermal treatment and methane production by mesophilic semi-continuous anaerobic digestion. A slightly modified version is presented in **Chapter 6**. *In preparation*.

7. **Cubero-Cardoso, J**., Gómez-Villegas, P., Santos-Martín, M., Sayago, A., Fernández-Recamáles, A., Borja, R., Fermoso, F.G., León, R., and Urbano, J. New antioxidant and

antimicrobial materials based on sulfur by inverse vulcanization reaction. A slightly modified version is presented in **Chapter 7**. *In preparation*.

8. **Cubero-Cardoso, J.**, Trujillo-Reyes, Á., Marín-Ayllón, P., Rodríguez-Gutiérrez, G., Villa-Gomez, D., Serrano, A., Borja, R., and Fermoso, F.G. 2020. Solubilization of phenols and sugars from raspberry extrudate by hydrothermal treatments. *Processes* 8(7): 1–16. MDPI. doi:10.3390/pr8070842.

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10. **Cubero-Cardoso, J**., Russo, E., Serrano, A., Trujillo-Reyes, Á., Villa-Gomez, D., Esposito, G., and Fermoso, F.G. Enhancing the recovery of volatile fatty acids from strawberry extrudate through anaerobic fermentation at different pH values. *Journal of Environmental Management*. *In preparation*.

11. Serrano, A., Russo, E., Chaves-Quesada, B., **Cubero-Cardoso, J.,** Trujillo-Reyes, Á., Esposito, G., and Fermoso, F.G. Recovery of volatile fatty acids from strawberry extrudate treated hydrothermal through anaerobic fermentation at different pH values. *In preparation*.

12. **Cubero-Cardoso, J.**, Braga, A.F.M., Trujillo-Reyes, Á., Serrano, A., Borja, R., and Fermoso, F.G. Effect of trace elements in anaerobic digestion of strawberry extrudate. *In preparation*.

13. **Cubero-Cardoso, J.**, Cuadri, A.A., Fermoso, F.G., Martín-Alfonso, J.E., and Urbano, J. Development and applications of novel polysulfides produced via inverse vulcanization based on vegetable oils and residual sulfur. *In preparation*.

14. **Cubero-Cardoso, J**., Haro-Martínez, A., Laura Galván, L., Sayago, A., Fernandez-Recámales, Fermoso, F.G., and Urbano, J. Efficient Hybrid Inorganic Polymer like adsorbent for Water Remediation. *In preparation*.

15. Santos-Martín, M., **Cubero-Cardoso, J**., Cortés-Triviño, E., Sayago, A., Urbano, J., and Fernandez-Recámales, A. Valorization of agro-food waste using NADES under mild conditions. *In preparation*.

16. Cortés-Triviño, E., **Cubero-Cardoso, J**., Fernández-Recámales, A., Sayago, A., Valencia, C., Urbano, J., and Franco, J.M. Greener alternatives to petroleum-based thickening formulations: The use of natural deep eutectic solvents (NADEs) and lignocellulosic materials. *In preparation*.

Chapter

Valorization Options of Strawberry Extrudate Agro-Waste. A Review

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Abstract

This review summarizes and critically analyzes the different types of potential valorization options for strawberry extrudate in order to have a broader overview of the potential management of this waste. Animal feed is commonly used as a management option for the strawberry extrudate; however, most of the strawberry extrudate is disposed in landfills. Strawberry extrudate contains different bioactive compounds that encourage the use of an alternative management approach than landfilled. The present review offers a complete comparative, including the advantages and drawbacks of each reviewed technique, to facilitate the selection of the most suitable technology for the different valorization scenarios. This review has been structured in three sections: 1. Composition of the strawberry extrudate and strawberry especially focused on their content in bioactive compounds. 2. The different techniques of extraction and purification of bioactive compounds. 3. The handling and management of the resulting biomass after the extraction process of bioactive compounds.

Keywords: strawberry extrudate, bioactive compounds, bioproducts, extraction techniques, purification

1. Introduction

In 2016, 8 million tons of strawberry were produced in the world with a value of agricultural gross production of 17,739 million US$ [1]. Besides its market as fresh product, strawberry is also used to produce many types of by-products, due to its peculiar flavor and aroma. Strawberry by-products are mainly formulated from a strawberry concentrate. The most common technology to obtain the strawberry concentrate is by extrusion. Strawberries are extruded by twin-screws up to several sieves with different mesh sizes. The sieves retain a residual fraction formed by the fibrous part and the achenes, named strawberry extrudate, which accounts about 7% of the manufactured strawberry [2].

Animal feed is commonly used as a management option for the strawberry extrudate, however, most of the strawberry extrudate is disposed in landfills, contributing to greenhouse emissions due to its high organic load [3]. Alternatives for strawberry extrudate management are required to avoid severe environmental

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Thermally-treated strawberry extrudate: A rich source of antioxidant phenols and sugars

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ABSTRACT

Strawberries have antioxidant, cardiovascular, and antiproliferative properties. The agroindustrial production of strawberry concentrate generates a food waste after extrudation that is usually landfilled. This strawberry extrudate is a rich source of valuable bioactive compounds such as phenols and sugars. In the present study, industrial thermal treatments currently in use for the valorization of other agricultural wastes were determined to be suitable for the treatment of strawberry extrudate. Thermal treatment conditions in the range of 90 °C to 200 °C were studied. Thermal treatment at 150 °C for 60 min without acid addition was the most efficient process based on the solubilization of sugars and phenols as well as the antioxidant capacity of the liquid phase produced. Instead of sending this residual fraction to landfill, such treatment would permit the use of strawberry extrudate as a source for the recovery of valuable bioactive compounds.

1. Introduction

The global strawberry industry produced 11 million tons of strawberries in 2014. The United States is the world's major producer with 12.2% of world production, followed by Mexico, Turkey, and Spain (FAO, 2017). The strawberry is a popular seasonal fruit due to its unique flavour and aroma. The strawberry is considered a functional food that offers multiple health benefits, including antioxidant, cardiovascular, antihypertensive, and antiproliferative effects (Basu, Nguyen, Betts, & Lyons, 2014). These health effects are due to strawberry's unique combination of nutrients, phytochemicals, and fibres, which play a synergistic role in characterizing strawberries as a functional food (Basu et al., 2014). Moreover, strawberries are an important source of B-group vitamins, vitamin C, vitamin E, potassium, folic acid, carotenoids, and specific flavonoids, such as pelargonidin, quercetin and catechin (Giampieri et al., 2012) or the tiliroside that is a glycosidic flavonoid and possesses anti-inflammatory, antioxidant, anticarcinogenic and hepatoprotective activities (Goto et al., 2012).

The high antioxidant capacity of strawberries is mainly due to the presence of ascorbic acid, ellagitannins and anthocyanins (Basu et al., 2014). Anthocyanins give the fruit its characteristic red color. Anthocyanins present in the strawberry have been investigated and identified as cyanidin and pelargonidin glycosides (Cerezo, Cuevas, Winterhalter, Garcia-Parrilla, & Troncoso, 2010), and pelargonidin-3-glucoside is the main anthocyanin in the strawberry (Cerezo et al., 2010). Other polyphenols, such as glucosides and glucuronides of quercetin and kaempferol, are also present (Cerezo et al., 2010; Cruz-Atonio et al., 2010). There is particular interest in determining strawberries' ellagic acid content due to its possible chemopreventive effects (da Silva Pinto, Lajolo, & Genovese, 2008). This compound may exist in a free form, as a glycoside, or bound as glucose esterified ellagitannins, like agrimonin as a ellagitannin dimer (Maas, Galletta, & Stoner, 1991). It is also remarkable the content of ellagitanins with antioxidant and cancer chemopreventive activities that might contribute to health benefits in humans (Cerdá, Tomás-Barberán, & Espín, 2005). Other important group of phenols with bioactives properties are the tannins, divided into condensed tannins (proanthocyanidins) and hydrolyzable tannins (Skrovankova, Sumczynski, Mlcek, Jurikova, & Sochor, 2015).

In addition to strawberry production and commercialization, the strawberry sector includes the manufacture of derived products. For example, around 21% of the total production of strawberries is used for the elaboration of products such as yogurt, juices, jams, etc. (Serrano, 2015). These products are generally elaborated from a strawberry concentrate. During the industrial process to obtain the strawberry concentrate, strawberries are extruded by several sieves with different mesh sizes. The residual fraction formed of the fibrous part and the achenes is retained in the sieves and named strawberry extrudate. Strawberry extrudate represents 7% of the weight of processed strawberries (Serrano, Siles, Chica, & Martin, 2014). The strawberry extrudate contains most of the beneficial components found in the whole

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Regular article

Extraction of phenolic compounds and production of biomethane from strawberry and raspberry extrudates

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HIGHLIGHTS

- Biorefinery approach: bioactive compounds extraction and anaerobic digestion.
- High concentration of valuable compounds is extracted from different berry extrudates.
- High methane production is attained from anaerobic digestion of remaining biomass.
- The proposed biorefinery approach offers higher benefits than just anaerobic digestion.

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Valorization

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 2402 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.

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,

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High-Value-Added Compound Recovery with High-Temperature Hydrothermal Treatment and Steam Explosion, and Subsequent Biomethanization of Residual Strawberry Extrudate

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Abstract: This study was on the comparison of hydrothermal treatments at 170 °C (steam injection) and 220 °C (steam explosion) to solubilize the organic matter contained in residual strawberry extrudate, focusing on phenolic compounds that were susceptible to be extracted and on sugars. After the extraction step, the remaining strawberry extrudate phases were subjected to anaerobic digestion to generate biogas that would compensate the energy requirements of the suggested hydrothermal treatments and to stabilize the remaining waste. Hydrothermal treatment at 220 °C allowed the recovery of 2053 mg of gallic acid eq. per kg of residual strawberry extrudate. By contrast, after hydrothermal treatment at 170 °C, only 394 mg of gallic acid eq. per kg of residual strawberry extrudate was recovered. Anaerobic digestion processes were applied to the de-phenolized liquid phase and the solid phase together, which generated similar methane productions, i.e., around 430 mL CH_4/g volatile solids, after both 170 °C and 220 °C hydrothermal treatments. Considering the latest observation, hydrothermal treatment at 220 °C is a preferable option for the valorization of residual strawberry extrudate (RSE) due to the high solubilization of valuable phenolic compounds that can be recovered.

Keywords: mesophilic anaerobic digestion; hydrothermal treatments; valorization; strawberry extrudate; phenols

1. Introduction

The strawberry sector has seen exponential growth in recent years, reaching 8.3 million tonnes in 2018 [1]. The strawberry sector includes not only a direct marketing of fresh fruit but also obtaining by-products derived from strawberries, such as yogurts, juices, or jams [2]. These by-products of strawberries generate a waste called residual strawberry extrudate (RSE) that is currently dumped in landfill. RSE is formed by achenes, fibers, and part of the juice of strawberries that is retained and rejected during the extrusion of the fruits to obtain a strawberry concentrate. This waste as well as the strawberry contains a wide variety of interesting bioactive compounds, with high concentrations of nutrients and phytochemicals, which could be recovered [3,4]. The great variety of nutrients and phytochemicals that strawberry contains is of great interest in our diet because they are beneficial to avoid or prevent different cardiovascular, neurological, cancerous, and other diseases [4,5].

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Mesophilic Semi-Continuous Anaerobic Digestion of Strawberry Extrudate Pretreated with Steam Explosion

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Abstract: The production of strawberry concentrate produces a side stream after extrusion that is commonly landfilled. This strawberry extrudate (SE), of lignocellulosic character, contains valuable bioactive compounds such as sugars and phenols. Thermal treatments, such as steam explosion, are currently used for the valorisation of agricultural lignocellulosic wastes due to their ability to impact the structure of the lignocellulose and hemicellulose present in these wastes, favouring the disruption of fibrous material. Steam explosion has already been shown as a promising technology for phenol recovery from SE. Biogas is an additional valuable resource that might be produced from thermally pretreated and de-phenolised SE. This study assessed the influence of a steam-explosion pretreatment and the subsequent recovery of phenolic compounds from the long-term operation of a semi-continuous anaerobic digester of pretreated SE. The anaerobic digestion of SE steam exploded at 220 °C for 5 min and de-phenolised was stable at an OLR of 0.5 g of volatile solids (VS)/(L·d), which permitted a specific production rate of 135 ± 11 mL of CH_4/(g of VS d). The system was not able to operate at an OLR of 1 g of VS/(L·d), which resulted in a failure of the process. Despite the inhibition threshold of phenolic compounds not being achieved, the inhibition of the anaerobic digestion process at an OLR of 1 g of VS/(L·d) was most likely due to the overloading of the system. This was indicated by the accumulation of soluble organic matter and volatile fatty acids. The increase in the propionic acid concentration up to 1300 mg/L when operating at OLRs higher than 0.5 g of VS/(L·d) could be the main factor responsible for the inhibition. An economic evaluation showed that the proposed approach (steam explosion, phenol recovery, and anaerobic digestion) would offer positive benefits, taking into account the high phenolic recovery (0.90 g of gallic acid equivalents/kg of extrudate) and the low sales price of the phenol extract, i.e., EUR 0.610/g of gallic acid equivalents, needed to reach zero net profit.

Keywords: strawberry extrudate; anaerobic digestion; economic assessment; steam explosion; phenol recovery; valorisation

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