Bioresource Technology 385 (2023) 129432

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Microwave-assisted autohydrolysis of avocado seed for the recovery of antioxidant phenolics and glucose

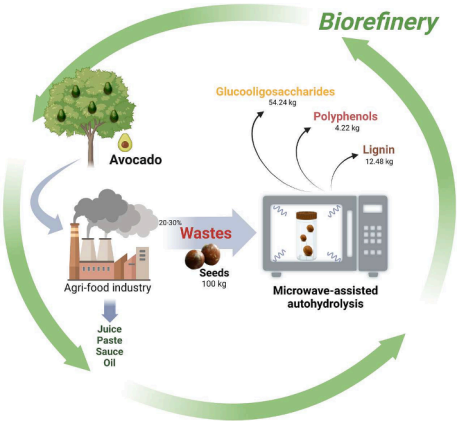
Alexandra Del-Castillo-Llamosas [a,b], Gemma Eibes [c], Pedro Ferreira-Santos [a], Alba Pérez-Pérez [a,b], Pablo G. Del-Río [a,b,d,*], Beatriz Gullón [a,b]

[a] Universidade de Vigo, Departamento de Enxeñaría Química, Facultade de Ciencias, 32004 Ourense, Spain
[b] Instituto de Agroecoloxía e Alimentación (IAA). Universidade de Vigo – Campus Auga, 32004 Ourense, España
[c] CRETUS, Department of Chemical Engineering, Universidade de Santiago de Compostela, Santiago de Compostela, 15706 A Coruña, Spain
[d] Stokes Laboratories, School of Engineering, Bernal Institute, University of Limerick, Limerick V94 T9PX, Ireland

HIGHLIGHTS

- Avocado seed biorefinery using microwave-assisted autohydrolysis (MAA) was planned.
- MAA (220 °C, 5 min) enabled recovery of high amount of phenolics and carbohydrates.
- Ethyl acetate extraction separated phenolic and carbohydrates in two streams.
- Spent solid and phenolic-free liquor were subjected to enzymatic hydrolysis.
- 90% of initial glucan was transformed into glucose in this strategy (54.47 per 100 kg avocado seed).

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Antioxidant phenolics
Avocado seeds
Biorefinery
Fermentable sugars
Microwave hydrothermal treatment

ABSTRACT

This study describes the valorization of avocado seed (AS) within a green biorefinery concept using microwave-assisted autohydrolysis. After the treatment at temperatures of 150–230 °C for 5 min, the resulting solid and liquor were characterized. The temperature of 220 °C led to the simultaneous optimal values of antioxidant phenolics/flavonoids (42.15 mg GAE/g AS, 31.89 RE/g AS, respectively) and glucose + glucooligosaccharides (38.82 g/L) in the liquor. Extraction with ethyl acetate allowed the recovery of the bioactive compounds while maintaining the polysaccharides in the liquor. The extract was rich in vanillin (99.02 mg/g AS) and contained several phenolic acids and flavonoids. The solid phase and the phenolic-free liquor were subjected to enzymatic hydrolysis to produce glucose, reaching values of 9.93 and 105 g glucose/L, respectively. This work demonstrates that microwave-assisted autohydrolysis is a promising technology to obtain fermentable sugars and antioxidant phenolic compounds from avocado seeds following a biorefinery scheme.

* Corresponding author.

https://doi.org/10.1016/j.biortech.2023.129432
Received 30 May 2023; Received in revised form 28 June 2023; Accepted 29 June 2023
Available online 30 June 2023

1. Introduction

Fresh fruit and vegetables are an important sector worldwide. In the European Union, 1.9% (3.4 million hectares) and 1.2% (2.2 million hectares) of all utilized agricultural land were dedicated to growing fruit and vegetables, respectively. Interestingly, in recent years, Spain has become a center for the production of subtropical/tropical fruits, including avocados (European Union, 2023).

Persea Americana Mill. (Avocado) is a plant originate in Mexico and Central America. Avocado fruits are within the most consumed fruits globally. In fact, the production of avocado has increased during the last decade, doubling its manufacture from 4 to 8 million tons (data from 2020) (FAOSTAT, 2022). Concomitantly, avocado has been gaining popularity among European farmers, and avocado orchards can now be found in Spain, Italy, Greece, and Portugal. In the case of Spain, each year its production is increasing (circa 90,000 tons by 2018), representing 95% of the manufacture in Europe; also its consumption increased exponentially (82,000 tons of avocado/year) (Confederation of British Industry, 2020).

Avocado has achieved significant interest and is usually catalogued as a "superfood", due to its nutritional composition, phytochemical content, and health benefits (Salazar-López et al., 2020). Industrial processing of avocado, for oil extraction and guacamole production, generates large quantities of "wastes", mainly peel and seed. Besides, the most abundant by-product is the seed, which can account for around 22–30% of the total fruit weight, representing 1.45 million tons per year that are wasted, producing a high generation or residue and increasing environmental pollution (Nyakang'i et al., 2023). Thus, it is important to reuse these by-products to create new economic models based on the "zero waste" movement. In this way, by reducing the negative impact of these biowastes on the environment and obtaining high-added value products (food additives, preservatives, antioxidants, nutraceuticals, cosmetics, etc.), it is possible to promote sustainability. In addition, based on a biorefinery framework, it is possible an integral valorization of food by-products, obtaining a wide range of bioproducts, for example, bioactive antioxidants, proteins and oligosaccharides, and glucose-derived polysaccharides that could be used for biofuels and/or building blocks production (Rodríguez-Martínez et al., 2022).

The avocado seed is particularly rich in bioactive compounds such as polysaccharides, proteins, vitamins, terpenoids and phenolic compounds (hydroxycinnamic acids, hydroxybenzoic acids, catechins, flavonols, and tannins) (Bangar et al., 2022; Rodríguez-Martínez et al., 2022). These phytochemicals play an important role in several biological and health-promoting processes, showing various bioactive actions like antioxidant, anticancer, antidiabetic, antimicrobial, anti-neurogenerative, and anti-inflammatory (Bangar et al., 2022).

Moreover, the methodologies used for avocado by-products managing is crucial to develop a viable biorefinery to obtain multiple products because they enable to selectively separate the main compounds for additional valorization. Hence, the need to obtain more ecological, sustainable and viable extraction processes has led to develop novel processes in full agreement with Green Chemistry principle (Ferreira-Santos et al., 2020). So far, several processes have been suggested to recover valuable components from avocado by-products, such as (i) conventional methods, like maceration, hydro-distillation, Soxhlet, and organic solvent extraction (ethanol, acetone, hexane…); and (ii) innovative "green" technologies, i.e., microwave-assisted extraction (MAE), ultrasounds, supercritical fluid extraction, and pressurized liquid extraction (Del Castillo-Llamosas et al., 2021; Rodríguez-Martínez et al., 2022). Among the used extraction methods, innovative methods show several advantages compared to conventional methods, such as higher efficiency, shorter processing times, selectivity, lesser solvent and energy consumption, and the possibility of recovering sensitive bioactive compounds (Ferreira-Santos et al., 2020).

Autohydrolysis treatment with water is considered an environmentally friendly method for the hydrolysis of biomass. However, a new advanced microwave heating technology is an alternative green method that partially overcomes the shortcomings of conventional autohydrolytic processes by direct conversion of electromagnetic energy into heat energy, provoking effective, selective and fast heating in the biomass and reaction medium (del Río et al., 2021). Conversely, from the hydrothermal point of view, water works both as a reaction medium and as a catalyst, proving to have excellent solubilization capacity to solubilize organic components, that aids the conversion of biomass (Gao et al., 2021). Within this framework, microwave-assisted autohydrolysis blends the benefits of water-based extractions and the positive effect of microwaves, and appears to be an effective alternative to manufacture high value products (bio-based compounds and materials) and energy (bio-based fuels), from different biomasses (Aguilar-Reynosa et al., 2017; del Río et al., 2021; Gao et al., 2021; Pérez-Pérez et al., 2023).

The main aim of this study was to valorize a highly produced food waste, such as avocado seed, following a biorefinery scheme. To accomplish that, microwave-assisted autohydrolysis was selected as extraction method and the chemical composition of the liquor (oligosaccharides, monosaccharides, phenolic and flavonoids content, and antioxidant capacity) and spent solid (polysaccharides) was determined. At selected conditions, the phenolic compounds were identified by HPLC-ESI, while the spent solid and the glucooligosaccharides-rich liquor were subjected to enzymatic hydrolysis to obtain fermentable sugars (glucose) with interest for biofuels and/or building blocks production.

2. Materials and methods

2.1. Materials, feedstock and chemical characterization

The reagents employed in this study were 2,2′-azino-di(3-ethylbenzo-thia-zoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-etramethylcrhoman-2-carboxylic acid (Trolox), arabinose, choline chloride, ethanol, Folin-Ciocalteu reagent, furfural, gallic acid, glucose, glycerol, hydrochloric acid, hydroxymethylfurfural, iron (III) chloride hexahydrate, methanol, potassium persulfate, rutin, sodium acetate trihydrate, sodium carbonate, sulfuric acid and xylose, that were purchased from Sigma-Aldrich (Barcelona, Spain). The enzymes Cellic CTec2, Termamyl® SC 4X and Saczyme® Yieldenzyme were kindly provided by Novozymes.

Avocado seed (AS) (Hass variety) from a local restaurant (Ourense, NW Spain) was used as raw material. AS was rinsed with tap water to eliminate avocado pulp and the layer that covered the seed. AS was milled to a smaller size than 1 mm after drying at room temperature. The obtained powder was collected in a zip bag and stored in a freezer (−20 °C).

AS was chemically analyzed following methods from NREL for ash (Sluiter et al., 2008b), ethanol extractives (Sluiter et al., 2008d), moisture (Sluiter et al., 2008a) and carbohydrate quantification of the extractives-free AS by quantitative acid hydrolysis (Sluiter et al., 2008c). The liquid phase obtained after the quantitative acid hydrolysis was filtrated and analyzed by HPLC (Agilent 1200 series, Palo Alto, CA, USA; injection of 5 μL of sample in a Rezex ROA Organic Acid H+ (Phenomenex) at 60 °C, 3 mM sulfuric acid mobile phase at 0.6 mL/min, refractive index detector at 35 °C) to quantify the amount of monosaccharides and organic acids. The liquid phase was also employed to calculate the uronic acids following the method by Blumenkrantz and Asboe-Hansen (1973). The solid phase was gravimetrically measured after dried in an oven for 24 h at 105 °C and quantified as Klason lignin. All the analytics were carried out in three replicates.

2.2. Microwave-assisted autohydrolysis (MAA)

A Monowave 450 single-mode microwave reactor (Anton Paar GmbH, Austria) was employed as equipment for microwave-assisted

autohydrolysis (MAA). Water and AS were introduced in a Pyrex vessel of 30 mL (G30) at a liquid-to-solid-ratio (LSR) of 15 mL of water/g of dry AS and heated up to the target temperature (150–230 °C) as fast as possible. Immediately after the objective temperature was attained, it was sustained for 5 min and let cool using an air compressor equipped to the microwave. The samples were magnetically stirred throughout the process and the temperature was determined using an infrared detector. The used energy in the heating stage was determined by the microwave reactor and employed for the quantitation of the energy consumption. The severity (S_0) of the process was quantified as a function of temperature and time employing the following equation (Overend et al., 1987):

$$S_0 = log\left(\int exp\left(\frac{T(t)-100}{14.75}\right)\cdot dt\right) \quad (1)$$

where S_0 is the severity, T(t) is the temperature during the heating and cooling stages, 100 °C is the reference temperature, and 14.75 °C is the typical value for the empiric parameter related to activation energy.

After MAA, the subsequent slurry was separated through filter paper, the liquid phase was collected and the solid phase was washed with water until reaching neutral pH, dried at room temperature and weighted to quantify the solid yield. Both phases were characterized as described in the following sections.

2.3. Total phenolic and flavonoid content, and antioxidant capacity of avocado seed liquor

AS liquor resulting from MAA was evaluated for phenolic and flavonoid quantification using the methods explained by Singleton and Rossi (1965) and Blasa et al., (2006), using gallic acid and rutin as standards and measured as gallic acid equivalent (GAE) and rutin equivalent (RE), respectively. The antioxidant capacity of AS liquor was also assayed by four methods: α,α-Diphenyl-β-picrylhydrazyl radical scavenging assay (DPPH), the 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid radical cation decolorization assay (ABTS) and the ferric reducing antioxidant power (FRAP) (Gullón et al., 2017) using Trolox as standard (Trolox equivalent, TE), and total antioxidant capacity (TAC) (Prieto et al., 1999) using ascorbic acid as standard (ascorbic acid equivalent, AAE). All the methods were performed in triplicates.

2.4. Chemical composition of the liquors and spent solids after microwave-assisted autohydrolysis

The liquor was filtered and injected in HPLC for monomers, acetic acid, hydroxymethylfurfural (HMF) and furfural quantification as described previously (section 2.3). Another aliquot of the liquor was subjected to acid posthydrolysis (sulfuric acid 4% w/w, 121 °C for 20 min), filtered and injected in HPLC to determine the amount of oligomers as a subtraction of sugars obtained from acid posthydrolysis and direct injection. Non-volatile compounds (NVC) were calculated after keeping an aliquot of the liquor in an oven 105 °C for 24 h and expressed as g of NVC/100 g raw avocado seed. All determinations were made in triplicate. Volatile compounds (VC) were calculated by difference with solid yield and non-volatile compounds.

The spent solid was chemically analyzed by quantitative acid hydrolysis as stated in section 2.1.

2.5. Phytochemical profile of phenolic compounds from avocado seed liquor using HPLC-ESI

In order to separate the phenolic compounds from avocado seed liquor, a liquid:liquid extraction with ethyl acetate (1:1 v/v) was performed. The mixture was agitated at room temperature during 15 min and poured in a decanting flask to be separated. The ethyl acetate fraction was recovered, and the aqueous fraction was subsequently extracted two more times with new ethyl acetate. The ethyl acetate fraction was then completely evaporated by a rotatory-evaporator at 40 °C. Methanol was used to resuspend the obtained sample, and an HPLC (Agilent 1260 series, Palo Alto, CA, USA) with AB SCIEX Triple Quad 3500 detector (AB Sciex, Foster City, CA, USA) and equipped with an electrospray source of ionization (ESI) was used for the determination of phytochemicals. A volume of injection of 5 μL was used, employing two mobile phases (formic acid 0.1% and acetonitrile with formic acid 0.1%) at a flow of 0.3 mL/min in a Luna C18 column (Phenomenex). A positive/negative source of ionization with turbo V™, with nitrogen as nebulizer and collision gas were employed. Multiple reaction monitoring (MRM) was used to obtain the data.

2.6. Enzymatic hydrolysis of solid and liquor of avocado seed

Spent solid and phenolic-free liquor after MAA at selected condition of 220 °C for 5 min were employed to produce glucose via enzymatic pathway using different enzymes. Firstly, the spent AS was blended with enzymes and water at a LSR of 20 g of biomass/g of liquid and Cellic CTec2 (enzymatic activity of 116 FPU/mL (Adney and Baker, 2008) to biomass ratio of 20 FPU/g. The temperature was set at 50 °C, agitation 200 rpm and the pH adjusted to 5 using 0.05 N citrate buffer. The saccharification time was set at 24 h.

For the case of the phenolic-free liquor, it was firstly concentrated 3 times via freeze-drying, reaching a concentration of glucose + glucooligosaccharides of around 115 g/L. After that, the commercial enzyme Termamyl® SC 4X (amylolytic activity of 78,405 UE/mL (Murado et al., 1993)) was added to the liquor at a ratio of 75 UE/g of liquor, adjusting the pH to 6 using citrate buffer 0.05 N. The mixture was submerged in a water bath at 90 °C for 3 h. Subsequently, the flask was cooled and a mixture of glucoamylase, acid acylase and cellulase, namely Saczyme® Yield (amylolytic activity of 12,507 UE/mL (Murado et al., 1993)), was added at a ratio of 300 UE/g liquor. The flask was placed in an orbital agitator at 50 °C, 200 rpm for 15 h.

After the saccharification of both spent solid and liquor was completed, the flasks were sampled, and after centrifugation and filtration, injected in HPLC at the same conditions explained in section 2.1 to quantify the glucose content. The glucose production after saccharification was also calculated in terms of glucose conversion (GC, %) taking into account the potential glucose. For the case of spent solid, it could be calculated by the following equation:

$$Gpot = Gn\cdot\frac{180}{162}\cdot\frac{\rho}{LSR+1-KL} \quad (2)$$

where Gn is the content of glucan in 100 g of spent avocado seed, 180/162 represents the stoichiometry for glucan hydration due to hydrolysis, ρ was selected as density for the medium (selecting 1005 g/L as average), LSR denotes the liquid-to-solid ratio, and KL denotes the content of Klason lignin in 100 g of spent avocado seed.

Alternatively, the potential glucose for the concentrated phenolic-free liquor was quantified by quantitative acid posthydrolysis (4% H_2SO_4, 121 °C, 20 min) to attain the maximum glucose content regarding monomers and oligomers of glucose.

2.7. Statistical analysis

Data resulting from TPC, TFC, ABTS, DPPH, FRAP, TAC and glucose + glucooligosaccharides (expressed as mean value ± standard deviation) were subjected to statistical analysis using software R (version 4.2.1). A one-way ANOVA (analysis of variance) followed by a Tukey's test was used to assay differences amongst samples, considering statistically differences at 95% of significance.

3. Results and discussion

3.1. Feedstock characterization

Chemical composition of avocado seed (AS) is displayed as follows, measured as g/100 g dry AS ± standard deviation: glucan, 57.33 ± 0.14; xylan, 9.71 ± 0.08; acetyl groups, 0.16 ± 0.00; Klason lignin, 8.25 ± 0.21; ethanol extractives, 12.89 ± 0.78; ash, 2.37 ± 0.05; uronic acids (galacturonic acid equivalents), 3.93 ± 0.25; others (by difference), 5.36.

AS is manly composed of polysaccharides (especially glucan), reaching a value of 67.04 g/100 g AS. A similar polysaccharide content was achieved by Araújo et al., (2020) with a value of 64.61 g/100 g AS. Conversely, other seeds in the literature accounted lower amount of polysaccharides. For instance, Oubannin et al., (2022) obtained 31.33 g/100 g from argan kernels and 51.33 g/100 g from black cumin oil seeds. Similarly, Hernández et al., (2014) characterized the olive stone reaching values for polysaccharides content of 50.20 g/100 g.

On the other hand, the content of lignin in AS is low, being similar to that reached in other food residues like potato peel (15.92 g/100 g) (Liang and McDonald, 2014). The ethanol extractives attained a value of 12.89 g/100 g AS, which is similar to that reported for olive stones (10.54 g/100 g) (Hernández et al., 2014).

3.2. Chemical characterization of the spent solid and liquor

After MAA processing at temperatures of 150–230 °C for 5 min (severities from 2.20 to 4.42), the resulting solid and liquor were chemically analyzed, and the main results are displayed in Table 1. Firstly, as expected, the energy consumption increased with the raise of the temperature in the process, varying from 11.64 to 24.03 MJ/kg of initial AS. Similar values were obtained when subjecting *Sargassum muticum* to MAA, with energy consumptions of 12.70 MJ/kg at $S_0 = 2.54$ (160 °C, 5.50 min) and 14.8 MJ/kg at $S_0 = 3.01$ (180 °C, 4 min) (del Río et al., 2021). In addition, Pérez-Pérez et al., (2023) processed *Robinia pseudoacacia* wood at 200 °C for 5 min ($S_0 = 3.71$) consuming 17.53 MJ/kg and at 230 °C for 2 min ($S_0 = 4.25$) consuming 20.28 MJ/kg, which are similar to the results achieved in the current work.

Regarding the fractionation data, the increase of temperature was intimately related to the increase in solubilization, which can be observed due to the decrease of the solid yield value from 53.15 to 17.26 g per 100 g of raw AS. In the same way, non-volatile compounds (i.e., carbohydrates solubilized in the liquor) increased from 36.10 g/100 g at 150 °C up to 71.60 g/100 g at 230 °C. On the other hand, the volatile compounds varied in a narrow range of 6.10–13.40 g/100 g, achieving higher values at harsher conditions, which can be translated to the generation of degradation volatile products such as acetic acid.

Regarding the spent solid, the main polysaccharide was glucan, varying in a range of 54.49–14.69 g glucan/100 g of spent solid. In this context, at milder conditions the glucan recovered in the spent solid was around 53% regarding initial glucan, while at the harshest conditions, the glucan retained in the spent solid was very scarce, only accounting for 5% of initial glucan. Similarly, the hemicelluloses (xylan and acetyl groups) were highly affected by the microwave processing, with values as low as 0.90 g xylan/100 g spent solid and 0.54 g acetyl groups/100 g spent solid. These values corresponded to a solubilization of up to 98% and 34% of initial xylan and initial acetyl groups, respectively, at $S_0 = 4.42$ (MAA at temperature of 230 °C). Dávila et al., (2016) also found that vine shoots processed by autohydrolyisis at 200 °C ($S_0 = 4.01$) resulted in 23% of glucan, 85% of xylan and 60% of acetyl groups were solubilized in the liquid phase.

Conversely, the lignin content increased with the raise of the

Table 1
Experimental conditions, energy consumption, fractionation data and composition of solid and liquor after microwave-assisted autohydrolysis of avocado seed. Different letters (glucose + glucooligosaccharides) indicate significant differences ($p < 0.05$).

T(°C)	150	160	170	180	190	200	210	220	230
S_0 (severity)	2.20	2.49	2.79	3.08	3.37	3.67	3.96	4.26	4.42
Energy consumed (MJ/kg)	11.64	12.63	13.41	14.40	15.74	17.19	19.01	19.76	24.03
Fractionation data (g/100 g of raw AS)									
Solid yield	53.15	41.35	34.97	30.57	26.50	20.85	17.26	18.01	19.32
Non-volatile compounds	36.10	48.57	58.93	58.09	62.08	66.20	70.37	68.59	71.60
Volatile compounds (by difference)	10.75	10.08	6.10	11.34	11.42	12.95	12.37	13.40	9.08
Spent solid composition (g/100 g of AS after MAA)									
Glucan	54.49 ± 0.38	52.41 ± 0.88	48.10 ± 0.35	46.20 ± 0.23	40.08 ± 0.09	29.85 ± 0.22	19.93 ± 0.42	17.98 ± 0.43	14.69 ± 0.33
Xylan	8.26 ± 0.27	5.13 ± 0.01	4.86 ± 0.06	3.96 ± 0.10	3.77 ± 0.03	3.40 ± 0.13	2.73 ± 0.01	2.12 ± 0.11	0.90 ± 0.06
Acetyl groups	0.69 ± 0.06	0.70 ± 0.05	0.70 ± 0.05	0.73 ± 0.00	0.74 ± 0.03	0.96 ± 0.04	0.94 ± 0.05	0.95 ± 0.00	0.54 ± 0.05
Klason lignin	22.71 ± 0.25	27.22 ± 0.09	31.29 ± 0.49	35.84 ± 0.51	42.30 ± 0.02	52.50 ± 1.01	65.56 ± 2.23	69.29 ± 1.31	78.48 ± 0.16
Liquor composition (g/L)									
Glucose	–	–	–	1.01	0.76	0.51	0.51	1.02	4.58
Xylose	–	–	–	5.96	6.05	5.76	5.38	5.24	5.66
Acetic acid	–	–	–	0.20	0.25	0.16	0.43	0.52	0.56
HMF	–	–	–	0.00	0.00	0.00	0.00	0.00	0.48
Furfural	–	–	–	0.00	0.00	0.00	0.00	0.00	0.00
Glucooligosaccharides	35.44 ± 0.16	31.56 ± 0.01	35.64 ± 1.94	36.01 ± 0.14	37.63 ± 3.10	40.43 ± 1.34	42.17 ± 0.49	37.80 ± 0.30	25.25 ± 0.18
Xylooligosaccharides	6.39 ± 0.42	7.05 ± 0.09	7.70 ± 0.53	1.97 ± 0.23	1.73 ± 0.47	2.21 ± 0.10	2.92 ± 0.04	2.66 ± 0.23	2.50 ± 0.04
Acetyl groups linked to oligosaccharides	0.22 ± 0.01	0.24 ± 0.03	0.20 ± 0.01	0.09 ± 0.02	0.04 ± 0.02	0.17 ± 0.02	0.00 ± 0.02	0.00 ± 0.00	0.06 ± 0.02
Glucose + glucooligosaccharides	35.44 ± 0.16^{bc}	31.56 ± 0.01^{ab}	35.64 ± 1.94^{bc}	37.02 ± 0.14 ^{cd}	38.39 ± 3.10^{ce}	40.94 ± 1.34^{de}	42.68 ± 0.49^{e}	38.82 ± 0.30^{ce}	29.83 ± 0.18^{a}

severity, reaching values as high as 78.48 g/100 g spent solid, which corresponded to lignin recoveries higher than 100% regarding initial lignin. This phenomenon has also been observed by other authors processing lignocellulosic materials by autohydrolysis, since the process triggers the formation of pseudolignin and the bond of lignin with carbohydrates that can be accounted as lignin (Sannigrahi et al., 2011).

On the other hand, the liquor after MAA was mainly composed of oligosaccharides, highlighting glucooligosaccharides which represented around 90% of the total of oligosaccharides (as average). The values of glucooligosaccharides grew with the increase of temperature up to 42.17 g/L at a temperature of 210 °C and decreased at higher temperatures with a minimum of 25.25 g/L at 230 °C triggering the formation of glucose (up to 4.58 g/L) and degradation products such as HMF (up to 0.48 g/L). The values of glucose + glucooligosaccharides were submitted to statistical tests. The results exhibited that the values obtained at temperatures of 200–220 °C were statistically different ($p < 0.05$) regarding those obtained at other temperatures. In this context, the highest values of glucose and glucooligosaccharides were attained amongst those temperatures, which may be selected in following steps of this biorefinery in order to valorize that fraction. Similarly, Araújo et al., (2020b) studied the optimization of starch extraction by microwave-assisted autohydrolysis, reaching a maximum of 47% at 180 °C for 30 min.

Regarding the xylooligosaccharides and acetyl groups linked to oligosaccharides, the values ranged 1.73–7.70 g/L and 0.00–0.24 g/L, respectively. Concerning the monomers, xylose varied in a narrow range of 5.24–6.05 g/L, whilst acetic acid was lower than 0.56 g/L.

3.3. Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant capacity assays (DPPH, ABTS, FRAP and TAC)

The resulting liquors of AS processed by MAA were analyzed to study the content in phenolics, flavonoids and antioxidant capacity, and the results are exhibited in Fig. 1.

Specifically, TPC varied in the range 9.24–59.60 mg GAE/g of AS, obtaining a higher content at the highest temperature (being significantly different at a 95% significance level). These values were in the range of those obtained by Segovia-Gómez et al., (2014) who employed 60% ethanol for 25 min at 93.64 °C to obtain a maximum value of 46.95 mg GAE/g AS. Alternatively, the results obtained in the current work were slightly lower than those obtained by Araújo et al., (2020a) (68.93 mg GAE/g of AS) when using microwave technology on avocado seed at conditions of 70 °C, 15 min and 50% ethanol v/v. Besides that, the positive effect of temperature becomes more plausible if the results of the current work are compared with those of Calderón-Oliver et al., (2016), who boiled the avocado seed for 30 min, obtaining around 5.70 mg GAE/g extract, while at the lower temperature of the current work (150 °C) 15.76 mg GAE/g extract were obtained, reaching a maximum of 81.43 mg GAE/g extract at the highest temperature (230 °C).

Regarding TFC, the results ranged 16.27–34.19 mg RE/g of AS, reaching maximal values of 31.39–34.19 mg RE/g of AS at temperatures of 200–220 °C, reporting no significant differences (p greater than 0.05), but however decreasing to a value of 28.29 mg RE/g of AS at a temperature of 230 °C. A similar trend was observed by Gullón et al., (2018) after the hydrothermal processing of chestnut shells. This phenomenon might be due to degradation reactions taking place at higher temperatures of autohydrolysis. In that case, a temperature of autohydrolysis of 180 °C ($S_0 = 3.08$) enabled the maximum recovery of flavonoids with a value of 38.56 mg RE/g. The flavonoids content obtained in the current work can also be positively compared to those obtained from other industrial food residues such as avocado seed extracted with ethanol 60% (Weremfo et al., 2020) or eggplant peels (Manousaki et al., 2016).

The antioxidant capacity of the liquors was determined by four different methods ABTS, DPPH, FRAP and TAC. Regarding ABTS, the values ranged 16.52–88.23 mg TE/g of AS, growing with the increase of temperature. When using pure methanol or ethanol 50% v/v on AS for 24 h at 4 °C, the antioxidant capacity of the obtained liquors, measured by ABTS, reached 30.97 and 65.97 mg TE/g AS, which are slightly lower than that obtained in this work (Segovia et al., 2018). Values in the same range were also assessed by del Río et al., (2021b) when subjecting *Sargassum muticum* to microwave hydrothermal processing, reaching up to 47.28 mg TE/g (antioxidant capacity determined by ABTS radical scavenging) at 180 °C for 11 min corresponding to $S_0 = 3.41$, while in the current work $S_0 = 3.37$ enabled 39.57 mg TE/g AS.

In contrast, DPPH reached values from 7.31 mg TE/g of AS at the mildest condition up to 50.67 mg TE/g of AS at the harshest. Similar values of DPPH (compared to the current work) were obtained when subjecting avocado peels to autohydrolysis with a maximum value of 53.9 mg TE/g of avocado peel at a temperature of 155 °C ($S_0 = 2.28$) (Del Castillo-Llamosas et al., 2021).

In addition, for the antioxidant capacity measured by FRAP, values from 9.64 mg TE/g of AS at 150° C up to 76.92 mg TE/g of AS at 230 °C were obtained. Comparatively, a liquor from AS extracted with pure methanol and ethanol 50% v/v (24 h, 4 °C) reached values of 79.24 and

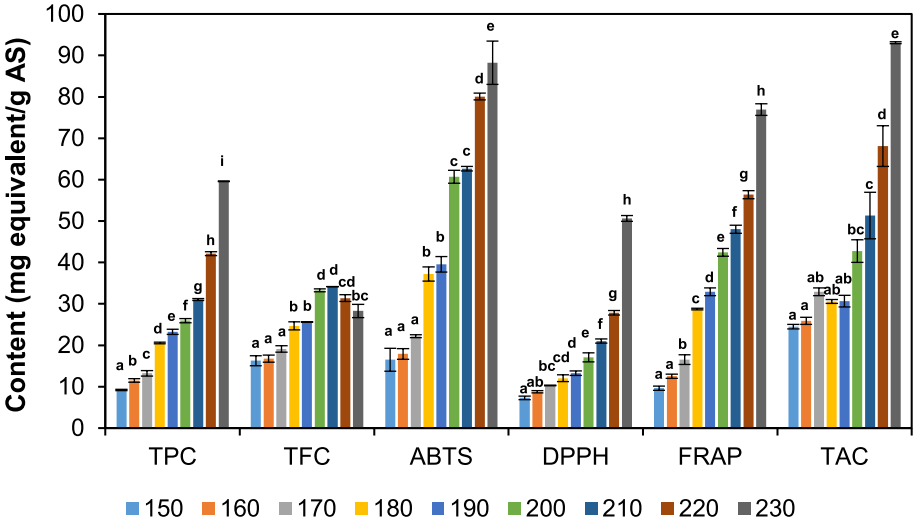


Fig. 1. Content of phenolics (TPC, gallic acid equivalents-GAE) and flavonoids (TFC, rutin equivalent-RE) and antioxidant capacity (ABTS, DPPH, FRAP (Trolox equivalents-TE) and TAC (ascorbic acid equivalents-AAE)) of avocado seed liquors after MAA at temperatures of 150 to 230 °C (S_0 of 2.20–4.42). Different letters imply significant differences at a 95% of confidence ($p < 0.05$).

109.85 mg TE/g AS, which are in the range of the results of the current study (Segovia et al., 2018). Calderón-Oliver et al., (2016) subjected AS to water extraction at boiling temperature for 30 min, and the resulting liquor presented an antioxidant capacity (measured as FRAP) of 9.50 mg TE/g extract, which can be positively compared to those obtained in the current study ranging 16.45–110.74 mg TE/g extract. On the other hand, liquor from *Robinia pseudoacacia* wood subjected to microwave-assisted autohydrolysis at 230 °C for 0.25 min (S_0 = 3.79) achieved slightly higher FRAP values of 102.30 mg TE/g (Pérez-Pérez et al., 2023).

Finally, TAC values reached 24.48–93.08 mg AAE/g of AS. In comparison, avocado from Hass variety subjected to ethanol (80% v/v) extraction for 12 h at 4 °C resulted in a liquor with up to 27.49 mg AAE/g (antioxidant capacity measured by TAC) (Lyu et al., 2023).

In summary, values measured by TPC, ABTS, DPPH, FRAP and TAC were higher (and significantly different at 95% confidence) at the harshest conditions. The only exception was TFC, which highest values were obtained at 200–220 °C (significantly different at 95% confidence). Taking into account the values obtained of TPC, TFC, antioxidant capacity and glucose + glucooligosaccharides, after statistical analysis, the condition of 220 °C for 5 min was selected as optimum to obtain the highest values of antioxidant phenolics/flavonoids and glucose + glucooligosaccharides.

3.4. Identification of phytochemicals

The phenolic compounds from the liquor were separated by an ethyl acetate liquid–liquid extraction and analyzed by HPLC-MS-ESI, identifying two flavonoids, two phenolic aldehydes and four phenolic acids. The main results are exhibited in Table 2.

Other authors have found some of these chemicals after extraction of avocado seed. For instance, convective drying procedure to maintain functional compounds was studied by Saavedra et al., (2017), discovering around 1.17 mg of ferulic acid in 100 g of AS. Similarly, Razola-Síaz et al., (2023) investigated the effect of lactic acid bacteria fermentation in the improving the extraction bioactive compounds from avocado seed, identifying isomers of luteolin/kaempferol and quercetin-diglucoside. On the other hand, hexane and ethanol were employed to extract phytochemicals from avocado seed, discovering the presence of vanillin, quercetin, Luteolin 7-O-(2″-O-pentosyl)hexoside (da Silva et al., 2022). It is important to highlight the presence of phthalic acid which may play a significant role in some biological properties such as antioxidant and antibacterial activities (Arulkumar et al., 2018).

Additionally, extracts from avocado peel also presented some of the compounds found in avocado seed extracts. For instance, Del-Castillo-Llamosas et al., (2023) discovered up to 0.15 mg of ferulic acid per g of avocado peel after extraction with deep eutectic solvents. In the same line, ferulic acid, *p*-coumaric acid, quercetin, vanillin and luteolin 7-O-(2″-O-pentosyl)hexoside were identified in autohydrolyzed liquors of avocado peel (Del Castillo-Llamosas et al., 2021). Moreover, some of these compounds could also be found in other agricultural wastes such as olive pomace extracted by water extraction by conventional heating (83 °C, 30 min), accounting for 0.21 mg *p*-coumaric acid/g, 0.24 mg ferulic acid/g, and 2.06 mg quercetin/g (Quero et al., 2022).

Table 2
Phytochemical profile of the main phenolic compounds identified in the liquor of AS after MAA at 220 °C (S_0 = 4.26).

Compound	Type	Content (mg/g AS)
luteolin	Flavonoid	1.30
quercetin	Flavonoid	0.97
ethylvainillin	Phenolic aldehyde	0.19
vanillin	Phenolic aldehyde	99.02
phthalic acid	Phenolic acid	1.21
ferulic acid	Phenolic acid	0.24
salycilic acid	Phenolic acid	0.78
p-coumaric acid	Phenolic acid	2.78

3.5. Glucose production from spent solid and liquor of avocado seed by enzymatic hydrolysis

After the separation of the phenolic compounds by liquid:liquid extraction with ethyl acetate, the aqueous solution obtained was still rich in oligomers and monomers of glucose. In this sense, two enzymatic saccharification processes of both solid and liquid was proposed to valorize the remaining carbohydrate fraction. To obtain a higher concentration of glucose, the phenolic-free liquor was freeze-dried and resuspended in water to get a three-fold higher concentration than the initial liquor, reaching 115 g glucose + glucooligosaccharides/L.

Regarding the saccharification of the spent solid, 100% of glucose conversion was attained at 24 h, with a final concentration of 9.93 g glucose/L. These results are in accordance with the results obtained after enzymatic saccharification of other spent solids coming from autohydrolysis processes. In this sense, *Sargassum muticum* processed via MAA at 180 °C for 11 min (S_0 = 3.41) reached a glucose conversion of 85% (around 11 g/L) after 9 h of enzymatic susceptibility assay (del Río et al., 2021). In the same line, Jesus et al., (2017) reached a glucose conversion of around 90% after autohydrolysis pretreatment of vine pruning at S_0 = 4.90.

On the other hand, the saccharification of the concentrated phenolic-free liquor yielded 105 g glucose/L, corresponding to 91.35% of glucose conversion at 15 h. These results may be positively compared to those attained by the authors in a previous study, when avocado seed was subjected to deep eutectic solvents and subcritical water extraction, obtaining a glucose-rich stream of 55 g/L (95% of glucose yield) after 48 h of enzymatic saccharification (Del-Castillo-Llamosas et al., 2023).

3.6. Mass balance of the biorefinery

The processing performed in this study plays an important part in the development of integrated multi-product biorefineries following the bioeconomy concept. Recently, many authors assessed the sustainability of biorefinery systems taking into account the chemical and energy saving (Tsui et al., 2022). In this regard the treatment proposed here avoids the use of toxic chemicals, since only water is required to fractionate the biomass. Additionally, a microwave heating system may result in a faster, more efficient, and more environmentally friendly heating process, thereby reducing energy consumption. In this context, the effect of different types of heating (microwaves and conventional heating) in aqueous media to produce oligosaccharides was studied by Dávila et al., (2021), implying a 71% of less energy consumption when employing microwaves. In addition, the optimization of an integrated biorefinery in terms of the energy balance and chemicals savings can benefit from the recent progress in modeling techniques, then contributing to the development of a circular bioeconomy (Tsui et al., 2023).

For that reason, the mass balance regarding the biorefinery scheme designed for avocado seed valorization producing fermentable sugars and antioxidant phenolics is depicted in Fig. 2. The mass balance of the whole process is shown, considering 100 kg of AS as feedstock. The first stage of microwave hydrothermal treatment at optimized conditions (220 °C, 5 min) enabled the recovery of a liquor highly rich in polysaccharides and antioxidant phenolic compounds. On the other hand, the spent solid, mainly constituted by lignin (69%), was subjected to an enzymatic hydrolysis, which allowed the complete recovery of its glucan content (3.6 kg of glucose) after 24 h of reaction. Additionally, the residual lignin could be employed not only as source for energy generation, but also to produce valuable fuels, chemicals and materials, that may enhance the economic feasibility of the biorefinery (Narron et al., 2016).

Antioxidant phenolics were recovered from the liquor through an extraction with ethyl acetate (1:1) since aromatic compounds tend to partition to the organic phase while maintaining polysaccharides in the

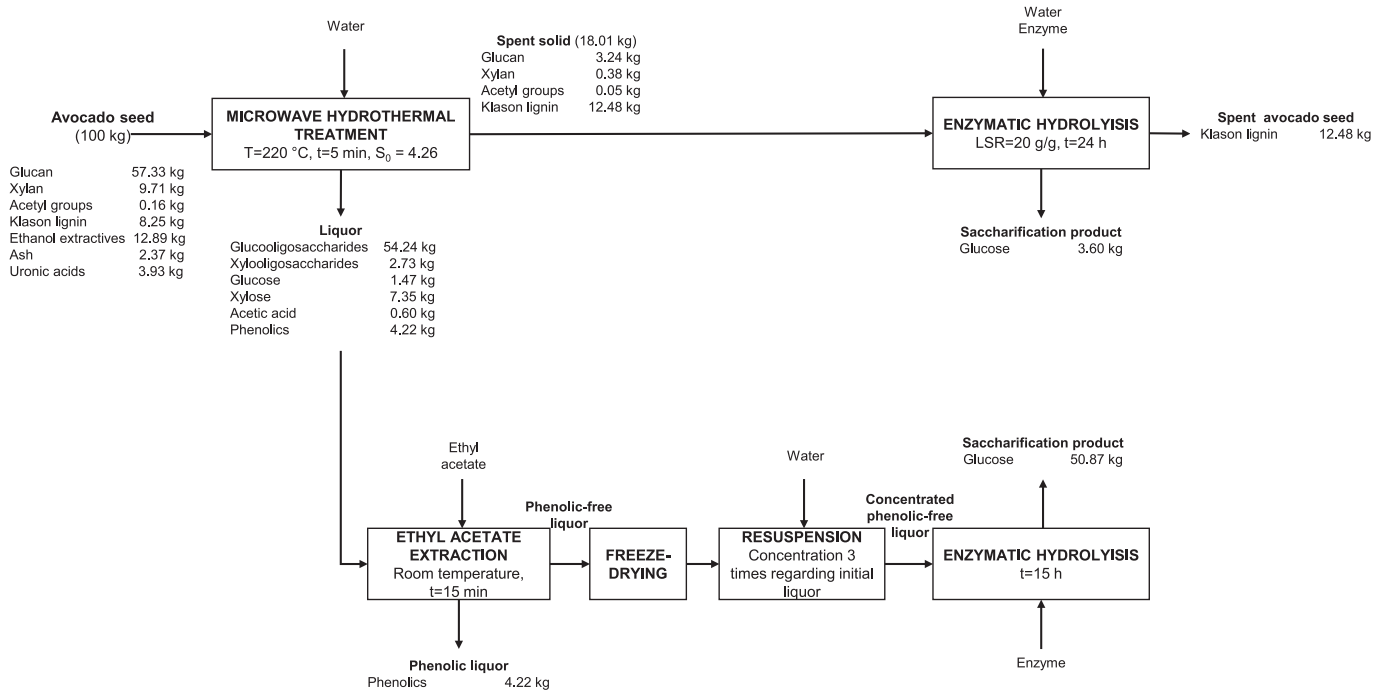


Fig. 2. Mass balance of the biorefinery proposed in this study, regarding kg of component per 100 kg of avocado seed in dry basis.

aqueous phase. After separation of both aqueous and organic phase, ethyl acetate was recovered by evaporation and could be reused in multiple cycles, then reducing the operational costs of the biorefinery through mass integration. The total phenolic content recovered amounted to a total of 4.22 kg of gallic acid equivalents, with high antioxidant activity, as demonstrated by the DPPH, ABTS, FRAP and assays. Concerning the composition of the extract, its major compound is vanillin, with a concentration of 6.26 mg/L, and contains other bioactive compounds such as flavonoids and phenolic acids.

The aqueous phenolic-free stream, rich in polysaccharides was concentrated three times and then subjected to an enzymatic hydrolysis stage. This step allowed to recover 50.87 kg of glucose (91.35% conversion of glucan to glucose) after 15 h of enzymatic treatment.

Summarizing, this biorefinery strategy produced an extract with 4.22 kg of gallic acid equivalents with high antioxidant activity, and a total of 54.47 kg of glucose, recovering more than 90% of the glucan present in the feedstock. If this glucose was intended for biofuel production, 27.83 kg of ethanol may be attained from 100 kg of AS. The high recovery of the two products obtained, indicates that the avocado seed is a very suitable raw material to obtain bioactive compounds in comparison with other feedstock. As for instance, similar strategies based on microwave-assisted autohydrolysis would allow the production of 15.9 kg of ethanol per 100 kg of corncob (Aguilar-Reynosa et al., 2017) and 5.34 kg of ethanol per 100 kg of *Sargassum muticum* (del Río et al., 2021).

4. Conclusions

Microwave-assisted autohydrolysis pretreatment is a promising technology to obtain fermentable sugars and antioxidant phenolic compounds from avocado seeds. Bioactive compounds with high antioxidant capacity were extracted from the liquor with ethyl acetate. The enzymatic hydrolysis of both the solid phase and the phenolic-free liquor allowed the recovery of more than 90% of the glucan in the raw material, with a total of 54.47 kg of glucose per 100 kg of avocado seed. Avocado seed processed by the proposed strategy enabled an integral valorization of this food by-product, diminishing the environmental issues and enhancing the economic profitability within a biorefinery framework.

CRediT authorship contribution statement

Alexandra Del-Castillo-Llamosas: Formal analysis, Investigation, Visualization, Writing – review & editing. **Gemma Eibes:** Validation, Visualization, Writing – review & editing. **Pedro Ferreira-Santos:** Writing – original draft, Visualization, Writing – review & editing. **Alba Pérez-Pérez:** Formal analysis, Investigation, Visualization, Writing – review & editing. **Pablo G. Del-Río:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Visualization, Writing – review & editing. **Beatriz Gullón:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Acknowledgments

Authors are grateful to the University of Vigo and CISUG for the financial support of Open Access publication, and to Consellería de Cultura, Educación e Ordenación Universitaria (Xunta de Galicia) through the contract ED431C 2017/62-GRC to Competitive Reference Group BV1 and through Project ED431F 2020/03, programs partially funded by FEDER. Pablo G. Del-Río would like to express gratitude to Consellería de Cultura, Educación, Formación Profesional e Universidades (Xunta de Galicia) for his postdoctoral grant (ED481B-2022-020). Authors would like to express gratitude to the Ministry of Science, Innovation and Universities of Spain for the FPU grant of Alba Pérez-Pérez (reference FPU21/02446), JDLC grant of Pedro Miguel Ferreira-

Santos (reference FJC2021-046978-I), RYC grant of Gemma Eibes (reference RYC2018-024846-I) and RYC grant of Beatriz Gullón (reference RYC2018-026177-I).

References

Adney, B., Baker, J., 2008. Measurement of Cellulase Activities: Laboratory Analytical Procedure (LAP). NREL (National Renewable Energy Laboratory). Technical report: NREL/TP-510-42628,.

Aguilar-Reynosa, A., Romaní, A., Rodríguez-Jasso, R.M., Aguilar, C.N., Garrote, G., Ruiz, H.A., 2017. Comparison of microwave and conduction-convection heating autohydrolysis pretreatment for bioethanol production. Bioresour. Technol. 243, 273–283. https://doi.org/10.1016/j.biortech.2017.06.096.

Araújo, R.G., Rodriguez-Jasso, R.M., Ruiz, H.A., Govea-Salas, M., Pintado, M.E., Aguilar, C.N., 2020a. Process optimization of microwave-assisted extraction of bioactive molecules from avocado seeds. Ind. Crops Prod. 154, 112623 https://doi.org/10.1016/j.indcrop.2020.112623.

Araújo, R.G., Rodríguez-Jasso, R.M., Ruiz, H.A., Govea-Salas, M., Rosas-Flores, W., Aguilar-González, M.A., Pintado, M.E., Lopez-Badillo, C., Luevanos, C., Aguilar, C. N., 2020b. Hydrothermal-microwave processing for starch extraction from Mexican avocado seeds: operational conditions and characterization. Processes 8. https://doi.org/10.3390/pr8070759.

Arulkumar, A., Rosemary, T., Paramasivam, S., Rajendran, R.B., 2018. Phytochemical composition, in vitro antioxidant, antibacterial potential and GC-MS analysis of red seaweeds (*Gracilaria corticata* and *Gracilaria edulis*) from Palk Bay. India. Biocatal. Agric. Biotechnol. 15, 63–71. https://doi.org/10.1016/j.bcab.2018.05.008.

Bangar, S.P., Dunno, K., Dhull, S.B., Kumar Siroha, A., Changan, S., Maqsood, S., Rusu, A. V., 2022. Avocado seed discoveries: chemical composition, biological properties, and industrial food applications. Food Chem. X 16, 100507. https://doi.org/10.1016/j.fochx.2022.100507.

Blasa, M., Candiracci, M., Accorsi, A., Piacentini, M.P., Albertini, M.C., Piatti, E., 2006. Raw Millefiori honey is packed full of antioxidants. Food Chem. 97, 217–222. https://doi.org/10.1016/j.foodchem.2005.03.039.

Blumenkrantz, N., Asboe-Hansen, G., 1973. New method for quantitative determination of uranic acids. Anal. Biochem. 54, 484–489. https://doi.org/10.1016/0003-2697(73)90377-1.

Calderón-Oliver, M., Escalona-Buendía, H.B., Medina-Campos, O.N., Pedraza-Chaverri, J., Pedroza-Islas, R., Ponce-Alquicira, E., 2016. Optimization of the antioxidant and antimicrobial response of the combined effect of nisin and avocado byproducts. Lwt 65, 46–52. https://doi.org/10.1016/j.lwt.2015.07.048.

Confederation of British Industry, C., 2020. The Spanish market potential for fresh fruit and vegetables [WWW Document]. URL https://www.cbi.eu/.

da Silva, G.G., Pimenta, L.P.S., Melo, J.O.F., de Mendonça, H.O.P., Augusti, R., Takahashi, J.A., 2022. Phytochemicals of avocado residues as potential acetylcholinesterase inhibitors, antioxidants, and neuroprotective agents. Molecules 27, 1892. https://doi.org/10.3390/molecules27061892.

Dávila, I., Gordobil, O., Labidi, J., Gullón, P., 2016. Assessment of suitability of vine shoots for hemicellulosic oligosaccharides production through aqueous processing. Bioresour. Technol. 211, 636–644. https://doi.org/10.1016/j.biortech.2016.03.153.

Dávila, I., Gullón, P., Labidi, J., 2021. Influence of the heating mechanism during the aqueous processing of vine shoots for the obtaining of hemicellulosic oligosaccharides. Waste Manag. 120, 146–155. https://doi.org/10.1016/j.wasman.2020.11.014.

Del Castillo-Llamosas, A., Rodríguez-Martínez, B., del Río, P.G., Eibes, G., Garrote, G., Gullón, B., 2021. Hydrothermal treatment of avocado peel waste for the simultaneous recovery of oligosaccharides and antioxidant phenolics. Bioresour. Technol. 342, 125981 https://doi.org/10.1016/j.biortech.2021.125981.

del Razola-Síaz, M.C., De Montijo-Prieto, S., Aznar-Ramos, M.J., Jiménez-Valera, M., Ruiz-Bravo, A., Verardo, V., Gómez-Caravaca, A.M., 2023. Effect of lactic acid bacteria fermentation on the polar compounds content with antioxidant and antidiabetic activity of avocado seed extracts. Fermentation 9, 42. https://doi.org/10.3390/fermentation9050420.

del Río, P.G., Gullón, B., Romaní, A., Garrote, G., 2021. Microwave hydrothermal processing of the invasive macroalgae *Sargassum muticum* within a green biorefinery scheme. Bioresour. Technol. 340, 125733 https://doi.org/10.1016/j.biortech.2021.125733.

Del-Castillo-Llamosas, A., Rodríguez-Rebelo, F., Rodríguez-Martínez, B., Mallo-Fraga, A., Del-Río, P.G., Gullón, B., 2023. Valorization of avocado seed wastes for antioxidant phenolics and carbohydrates recovery using deep eutectic solvents (DES). Antioxidants 12, 1156. https://doi.org/10.3390/antiox12061156.

FAOSTAT, 2022. Food and Agriculture Organization of the United Nations [WWW Document]. URL http://faostat3.fao.org.

Ferreira-Santos, P., Zanuso, E., Genisheva, Z., Rocha, C.M.R., Teixeira, J.A., 2020. Green and sustainable valorization of bioactive phenolic compounds from *Pinus* by-products. Molecules 25, 2931. https://doi.org/10.3390/molecules25122931.

Gao, Y., Remón, J., Matharu, A.S., 2021. Microwave-assisted hydrothermal treatments for biomass valorisation: a critical review. Green Chem. 23, 3502–3525. https://doi.org/10.1039/d1gc00623a.

Gullón, B., Eibes, G., Moreira, M.T., Dávila, I., Labidi, J., Gullón, P., 2017. Antioxidant and antimicrobial activities of extracts obtained from the refining of autohydrolysis liquors of vine shoots. Ind. Crops Prod. 107, 105–113. https://doi.org/10.1016/j.indcrop.2017.05.034.

Gullón, B., Eibes, G., Dávila, I., Moreira, M.T., Labidi, J., Gullón, P., 2018. Hydrothermal treatment of chestnut shells (*Castanea sativa*) to produce oligosaccharides and antioxidant compounds. Carbohydr. Polym. 192, 75–83. https://doi.org/10.1016/j.carbpol.2018.03.051.

Hernández, V., Romero-García, J.M., Dávila, J.A., Castro, E., Cardona, C.A., 2014. Techno-economic and environmental assessment of an olive stone based biorefinery. Resour. Conserv. Recycl. 92, 145–150. https://doi.org/10.1016/j.resconrec.2014.09.008.

Jesus, M.S., Romaní, A., Genisheva, Z., Teixeira, J.A., Domingues, L., 2017. Integral valorization of vine pruning residue by sequential autohydrolysis stages. J. Clean. Prod. 168, 74–86. https://doi.org/10.1016/j.jclepro.2017.08.230.

Liang, S., McDonald, A.G., 2014. Chemical and thermal characterization of potato peel waste and its fermentation residue as potential resources for biofuel and bioproducts production. J. Agric. Food Chem. 62, 8421–8429. https://doi.org/10.1021/jf5019406.

Lyu, X., Agar, O.T., Barrow, C.J., Dunshea, F.R., Suleria, H.A.R., 2023. Phenolic compounds profiling and their antioxidant capacity in the peel, pulp, and seed of australian grown avocado. Antioxidants 12, 185. https://doi.org/10.3390/antiox12010185.

Manousaki, A., Jancheva, M., Grigorakis, S., Makris, D.P., 2016. Extraction of antioxidant phenolics from agri-food waste biomass using a newly designed glycerol-based natural low-transition temperature mixture: a comparison with conventional eco-friendly solvents. Recycling 1, 194–204. https://doi.org/10.3390/recycling1010194.

Murado, M.A., Gonzalez, M.P., Pastrana, L., Siso, M.I.G., Miron, J., Montemayor, M.I., 1993. Enhancement of the pioproduction potential of an amylaceous effluent. Bioresour. Technol. 44, 155–163.

Narron, R.H., Kim, H., Chang, H.M., Jameel, H., Park, S., 2016. Biomass pretreatments capable of enabling lignin valorization in a biorefinery process. Curr. Opin. Biotechnol. 38, 39–46. https://doi.org/10.1016/j.copbio.2015.12.018.

Nyakang'i, C.O., Ebere, R., Marete, E., Arimi, J.M., 2023. Avocado production in Kenya in relation to the world, Avocado by-products (seeds and peels) functionality and utilization in food products. Appl. Food Res. 3, 100275 https://doi.org/10.1016/j.afres.2023.100275.

Oubannin, S., Bijla, L., Gagour, J., Hajir, J., Aabd, N.A., Sakar, E.H., Salama, M.A., Gharby, S., 2022. A comparative evaluation of proximate composition, elemental profiling and oil physicochemical properties of black cumin (*Nigella sativa* L.) seeds and argan (*Argania spinosa* L. Skeels) kernels. Chem. Data Collect. 41, 100920 https://doi.org/10.1016/j.cdc.2022.100920.

Overend, R.P., Chornet, E., Gascoigne, J.A., 1987. Fractionation of lignocellulosics by steam-aqueous pretreatments. Philos. Trans. R. Soc. London. Ser. A. Math. Phys. Sci. 321, 523–536. https://doi.org/10.1098/rsta.1987.0029.

Pérez-Pérez, A., Gullón, B., Lobato-Rodríguez, Á., Garrote, G., del Río, P.G., 2023. Microwave-assisted extraction of hemicellulosic oligosaccharides and phenolics from Robinia pseudoacacia wood. Carbohydr. Polym. 301, 120364 https://doi.org/10.1016/j.carbpol.2022.120364.

Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal. Biochem. 269, 337–341. https://doi.org/10.1006/abio.1999.4019.

Quero, J., Ballesteros, L.F., Ferreira-Santos, P., Velderrain-Rodriguez, G.R., Rocha, C.M. R., Pereira, R.N., Teixeira, J.A., Martin-Belloso, O., Osada, J., Rodríguez-Yoldi, M.J., 2022. Unveiling the antioxidant therapeutic functionality of sustainable olive pomace active ingredients. Antioxidants 11, 1–22. https://doi.org/10.3390/antiox11050828.

Rodríguez-Martínez, B., Romaní, A., Eibes, G., Garrote, G., Gullón, B., del Río, P.G., 2022. Potential and prospects for utilization of avocado by-products in integrated biorefineries. Bioresour. Technol. 364, 128034 https://doi.org/10.1016/j.biortech.2022.128034.

Saavedra, J., Córdova, A., Navarro, R., Díaz-Calderón, P., Fuentealba, C., Astudillo-Castro, C., Toledo, L., Enrione, J., Galvez, L., 2017. Industrial avocado waste: functional compounds preservation by convective drying process. J. Food Eng. 198, 81–90. https://doi.org/10.1016/j.jfoodeng.2016.11.018.

Salazar-López, N.J., Domínguez-Avila, J.A., Yahia, E.M., Belmonte-Herrera, B.H., Wall-Medrano, A., Montalvo-González, E., González-Aguilar, G.A., 2020. Avocado fruit and by-products as potential sources of bioactive compounds. Food Res. Int. 138 https://doi.org/10.1016/j.foodres.2020.109774.

Sannigrahi, P., Kim, D.H., Jung, S., Ragauskas, A., 2011. Pseudo-lignin and pretreatment chemistry. Energy Environ. Sci. 4, 1306–1310. https://doi.org/10.1039/c0ee00378f.

Segovia, F.J., Hidalgo, G.I., Villasante, J., Ramis, X., Almajano, M.P., 2018. Avocado seed: A comparative study of antioxidant content and capacity in protecting oil models from oxidation. Molecules 23, 2421. https://doi.org/10.3390/molecules23102421.

Segovia-Gómez, F., Peirósánchez, S., Iradi, M.G.G., Azman, N.A.M., Almajano, M.P., 2014. Avocado seeds: extraction optimization and possible use as antioxidant in food. Antioxidants 3, 439–454. https://doi.org/10.3390/antiox3020439.

Singleton, V.L., Rossi, J.A.J., 1965. Colorimetry to total phenolics with phosphomolybdic acid reagents. Am. J. Enol. Vinic. 16, 144–158.

Sluiter, A., Hames, B., Hyman, D., Payne, C., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Wolfe, J., 2008a. Determination of total solids in biomass and total dissolved solids in liquid process samples. Laboratory Analytical Procedure (LAP), National Renewable Energy Laboratory. https://doi.org/NREL/TP-510-42621.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 2008b. Determination of ash in biomass. Laboratory Analytical Procedure (LAP), National Renewable Energy Laboratory. https://doi.org/NREL/TP-510-42619.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2008c. Determination of structural carbohydrates and lignin in biomass. Laboratory

Analytical Procedure (LAP), National Renewable Energy Laboratory. https://doi.org/NREL/TP-510-42618.
Sluiter, A., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 2008d. Determination of Extractives in Biomass. Laboratory Analytical Procedure (LAP), National Renewable Energy Laboratory. https://doi.org/10.1016/j.rmr.2016.02.006.
Tsui, T.H., Zhang, L., Zhang, J., Dai, Y., Tong, Y.W., 2022. Engineering interface between bioenergy recovery and biogas desulfurization: sustainability interplays of biochar application. Renew. Sustain. Energy Rev. 157, 112053 https://doi.org/10.1016/j.rser.2021.112053.
Tsui, T.H., van Loosdrecht, M.C.M., Dai, Y., Tong, Y.W., 2023. Machine learning and circular bioeconomy: building new resource efficiency from diverse waste streams. Bioresour. Technol. 369, 128445 https://doi.org/10.1016/j.biortech.2022.128445.
European Union, (EU), 2023. Eurostat [WWW Document]. URL https://ec.europa.eu/eurostat/.
Weremfo, A., Adulley, F., Adarkwah-Yiadom, M., 2020. Simultaneous optimization of microwave-assisted extraction of phenolic compounds and antioxidant activity of avocado (*Persea americana* Mill.) seeds using response surface methodology. J. Anal. Methods Chem. 2020, 1–11. https://doi.org/10.1155/2020/7541927.