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Sustainable management of wine-derived leftovers: Enhanced extraction of antioxidants and production of levulinic acid by microwave-assisted processing

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ABSTRACT

Within the framework of circular economy and process intensification, this work aimed to develop a two-way, microwave (MW)-assisted valorisation protocol for winemaking waste, namely grape stalk (GS), grape marc (GM) and exhausted grape marc (EGM). The first step evaluates the recovery of biologically active compounds (BACs). In this context, MW-assisted extraction (MAE) (200 W, 100 ºC, 2 h) demonstrated to be an intensified approach for processing EGM, enhancing the total antioxidant capacity (TAC) by up to 436% (TAC) compared to conventional soaking. Various polyphenols including flavonols (quercetin), flavanols (catequin, epicatechin), anthocyanins (procyanidins (B1-B7)), as well as glycosylated structures (i.e., kaempferol 3-O-glucoside), were detected by LC-MS analysis in this matrix. Simultaneously, the conversion to the bio-based chemical levulinic acid (LevA) was carried out. Considering the environmental factor involved in the dimension of sustainability, environmentally friendly practices were adopted, based on an aqueous single-phase without organic solvent, reusable catalysts as *p*-toluenesulfonic acid (*p*-TsA), and short reaction times (20 min). LevA yield reached 33.09% molar from the extractives-free (EF)-EGM matrix, surpassing the 22.31% obtained from the untreated one. Nonetheless, GS exhibited higher efficiency, yielding up to 34.75 and 59.83% from the untreated- and EF-GS matrices, respectively.

1. Introduction

Wine production is a major agricultural process worldwide, with an estimated production around 244 mhL in 2023, according to data from the International Organization of Vine and Wine [1]. However, a substantial challenge lies in the significant generation of organic residues during winemaking. Unfortunately, these by-products are often underutilised, contributing to environmental issues such as pollution and CO_2 emissions [2].

The imperative for alternative, renewable feedstocks to produce high-added-value chemicals has gained prominence in recent years. Concretely, for winemaking by-products, grape marc (GM) might be the most valuable matrix, alongside other significant materials as grape stalks (GS) and exhausted grape marc (EGM) [2–4]. While existing literature is predominantly focused on the recovery of biologically active compounds (BACs), such as the polyphenols present in these matrices [5,6], which show potential health benefits [7]. Furthermore, the valorisation of GM as a potential bioenergy source is also remarkable, with some recently published articles for GM and vine pruning residues [4,8].

Nonetheless, there is a noticeable lack of literature on the development of fine, industrial-applied chemicals from the fibre fraction

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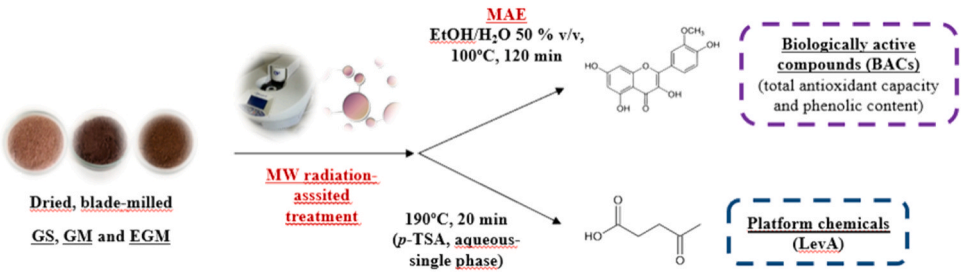

Fig. 1. Two-way valorisation for the winemaking waste grape stalks (GS), grape marc (GM) and exhausted grape marc (GM) via microwave (MW) radiation-assisted treatment: production of levulinic acid (LevA) with *p*-toluenesulfonic acid (*p*-TSA) and recovery of BACs through MW-assisted extraction (MAE).

(cellulose, hemicellulose, and even lignin) present in these waste. Limited studies, such as previous work on GS for the exploitation of lignin towards bioaromatics (for instance, vanillin or syringaldehyde) [9], and a preliminary exploration of levulinic acid (LevA) [10], highlight the potential of these by-products for chemical development. This study aims to fill this research gap by investigating the untapped potential in winemaking by-products, specifically GS, GM, and EGM, focusing on the development of fine chemicals from the fibre fraction of this kind of waste.

The significance of LevA as a versatile chemical building block lies in the wide range of applications that cover, for instance, the development of solvents and plastics [11], the production of fuels such as γ-valerolactone or ethyl levulinates [12], or the intermediary role in pharmaceutical sector in drug synthesis processes [13]. In summary, owing to its diverse applications and importance, LevA is considered as one of the top-10 bio-based chemicals derived from biomass, according to US Department of Energy [14]. Consequently, there is a critical need to explore its potential production from underutilised sources, such as vinification by-products.

Simultaneously, process intensification through innovative technologies offers an efficient and sustainable approach for upgrading to high-added-value chemicals. Notably, the recovery of BACs from winemaking by-products has been explored using methods such as microwaves (MW) [15], ultrasonication (US) [16,17] or pulsed electric fields (PEF) [18,19]. Microwave-assisted extraction (MAE) is particularly effective for the recovery of BACs from plant biomass due to the water content in cells that enhances microwave energy absorption. This energy-efficient process involves the penetration of electromagnetic waves into the matrix, resulting in elevated temperature and water vapor pressure inside the cell. This subsequently leads to the cell-wall rupture and the release of intracellular metabolites through a highly energy-saved process [20]. For synthesis purposes (for instance, towards LevA [10,21]), this energy saving translates to minimal side reactions and undesired by-products, with higher yields at shorter residence times compared to conventional methods [22]. These reasons make MW an environmentally friendly and efficient protocol that has found extensive application in diverse fields, ranging from organic synthesis and medicinal chemistry to biomass treatment [23].

Based on the provided background, the main objective of this study is the exploitation of the winemaking materials GS, GM and EGM through a two-way protocol enabled by MW, thus confirming the overall effectiveness of this non-conventional technology in various valorisation processes. The initial phase focused on the effective recovery of biologically active compounds (BACs), with EGM showcasing noteworthy activity. Following this, an environmentally sustainable processing was formulated for the production of LevA, which has been scarcely documented in the literature from these wine-derived materials, thus supposing this study an important advance in the state-of-the-art by addressing this research gap.

2. Material and methods

2.1. Chemicals

p-Toluenesulfonic acid (>98.5%, CAS No. 6192–52–5), potassium bromide (KBr) (CAS No. 7758–02–3), 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid sodium salt (98 atom % D, CAS No. 24493–21–8), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (CAS No. 53188–07–1), potassium persulfate ($K_2S_2O_8$) (CAS No. 7727–21–1), 2,2′-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) (CAS No. 30931–67–0), gallic acid (CAS No. 149–91–7), Folin–Ciocalteu reagent, fluorescein (CAS No. 2321–07–5), and 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) (CAS No. 2997–92–4) were purchased from Sigma-Aldrich (Steinheim, Baden-Württemberg, Germany). Deuterium oxide (D_2O) (99.96% atom D, CAS No. 7789–20–0), dimethyl sulfoxide (DMSO) (CAS No. 67–68–5), ethanol (CAS No. 64–17–5), sodium phosphate (Na_2HPO_4) (CAS No. 7601–54–9), sodium carbonate (Na_2CO_3) (CAS No. 497–19–8) and potassium phosphate (KH_2PO_4) (CAS No. 7 778–77–0) were acquired from VWR (Saint-Prix, France). Ethyl acetate (CAS No. 141–78–6) was supplied from Scharlab. Maleic acid (CAS No. 110–16–7) was obtained from Fluka.

2.2. Feedstock collection and pretreatment

Grape stalk (GS), grape marc (GM) and exhausted grape marc (EGM) were provided by ALVINESA NATURAL INGREDIENTS S.A. (Daimiel, Ciudad Real, Spain) following the 2017 harvesting season and stored at −4 ºC until use. For soaking experiments, they were defrosted and utilised without additional treatment. The moisture content in the raw residue was determined as follows: 76.35±1.20% for GS, 66.82±4.50% for GM and 7.75±0.35% for EGM. For microwave treatment (antioxidants and levulinic acid), the samples were initially lyophilized using a Telstar LyoQuest-85 PLUS freeze dryer (Telstar Technologies, Terrasa, Spain) and subsequently blade-milled at 20,000 rpm for 1 min (Fritsch Pulverisette 14, Idar-Oberstein, Germany). This process resulted in a homogeneous 1 mm-particle-size powder suitable for subsequent experiments.

2.3. Valorisation of winemaking-derived materials via microwave (MW) radiation

Fig. 1 summarises the protocol implemented in this study. The methodology for each valorisation approach and the experimental conditions selected are described below. Given the focus of this study on evaluating the overall effectiveness of the valorisation process, encompassing both the extraction of biologically active compounds (BACs) and the conversion to LevA via MW-assisted radiation (Fig. 1), detailed optimization of each individual step was not extensively pursued. Thus, previously established MW conditions, which yielded satisfactory results, were applied.

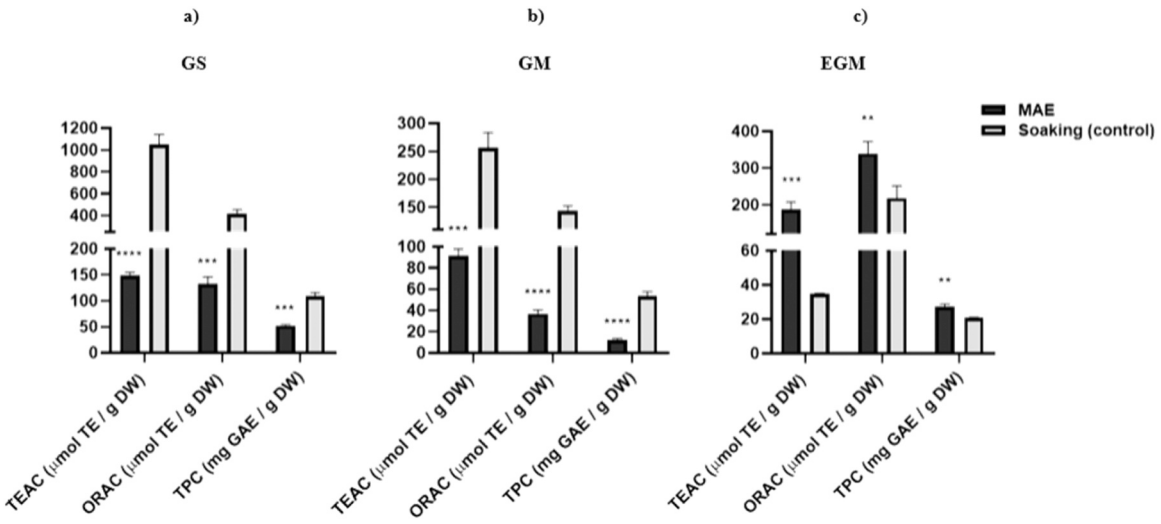

Fig. 2. Total Antioxidant Capacity (TEAC, ORAC) and Polyphenols Content (TPC) for grape stalks (GS) (a), grape marc (GM) (b), and exhausted grape marc (EGM) (c) after microwave-assisted extraction (MAE). Results are expressed as μmol Trolox Equivalent/g dry weight (μmol TE/g DW) for TEAC and ORAC, and as mg Gallic Acid Equivalents/g dry weight (mg GAE/g DW) for TPC. **** $p < 0.0001$; *** $p < 0.001$; $p < 0.01$ *vs.* control (soaking).

2.3.1. *Recovery of biologically active compounds*

2.3.1.1. Obtaining antioxidants-rich liquid extracts. Antioxidants-rich liquid extracts were obtained via microwave (MW)-assisted extraction (MAE) and conventional soaking, which was employed for comparative purposes.

The MAE treatment was carried out in a CEM Discover monomode MW reactor (CEM Discover SP, Matthews, NC, USA) operating in a closed mode. The extraction was conducted at 100 ºC for 120 min based on previous studies [24], aiming to assess the thermal effect of MWs and enhance process efficiency. Temperature control was facilitated by an infrared sensor. The maximum power was set at 200 W, and pressure ranged from 0 to 50 psi.

For MW-experiments, 1 g of dried, blade-milled solid was placed into a 35 mL MW glass reactor with 10 mL of an EtOH/H_2O mixture (50% v/v). After extraction, the crude was vacuum-filtered and thoroughly washed with the same EtOH/H_2O solution. The solid fraction was discarded, and the liquid fraction stored for subsequent antioxidant assays.

For soaking, a 500 mL Erlenmeyer flask was filled with 10 g of the raw, untreated matrix and 100 mL of the aforementioned EtOH/H_2O solution. The mixture was stirred for 24 h at room temperature (25ºC), following the conditions established by Lapornik et *al.* [25] for the extraction of BACs from different wine waste. Subsequent workup after extraction mirrored the process described above for MAE.

2.3.1.2. Antioxidant assays. Total antioxidant capacity (TAC) assessed through TEAC and ORAC assays, as well as total phenolic content (TPC) via Folin-Ciocalteu method, were evaluated following the methods reported in our prior studies [24, 26–27]. TAC was expressed as μmol Trolox Equivalent/g dry weight (μmol TE/g DW), and TPC as mg Gallic Acid Equivalents/g dry weight (mg GAE/g DW).

2.3.1.3. LC-MS analysis (phenolic profile). The phenolic profile for the EGM crude after MAE was determined similarly to previous works [24, 28].

Characterisation was performed using an Agilent 1260 Infinity (Agilent, Waldbronn, Germany), equipped with a Waters UPLC C18 column (1.7 μm, 2.1 × 50 mm, Acquity UPLC BEH.C18, Waters, Cerdanyola del Vallès, Spain) for separating the main phenolic compounds in the samples. Identification was carried out using a TripleTOF™ 5600 LC/MS/MS system (AB SCIEX, Foster City, CA, USA).

In a typical experiment, a mobile phase consisting of solvent A (water with 0.1% formic acid) and solvent B (methanol with 0.1% formic acid) was used with the following gradient: 0 min 90% A; 13 min 100% (B); 15 min 90% A. The injection volume was 5 μL at 0.4 mL/min flow rate. MS data were acquired in negative mode between 80 and 1200 *m/z*, and the IDA acquisition method utilized the survey scan type (TOF-MS) with dependent scan type (product ion). Main MS analysis parameters included ion spray voltage (−4500 V), declustering potential (90 V), collision energy (−50 V), a temperature of 400ºC with 25 psi curtain gas, and both ion source gas 1 (GC1) and ion source gas 2 (GS2) set at 50 psi. IDA MS/MS analysis employed an ion tolerance of 50 mDa, 25 V collision energy and activated dynamic background subtract. Data acquisition and processing were conducted using analyst PeakView1.1 software (AB SCIEX, Foster Molecules 2019, 24, 797 11 of 13 City, CA, USA), along with its applications (XIC Manager and Formula Finder).

Quantification was performed using a calibration curve, considering a representative polyphenol from some of the main groups: catechin (for flavanols), kaempferol (for flavonols), cyanidin (for anthocyanins), gallic acid (for phenolic acids) and apigenin (flavones).

2.3.2. *Production of levulinic acid (LevA)*

Experiments for the synthesis of LevA were conducted using MW radiation-assisted treatment, employing the same reactor as described above. The selection of the experimental conditions was based on previous research recently reported in literature [24,29]. In a standard assay, 100 mg of dried, blade-milled solid were placed in a 10 mL MW glass vial. The reactor was then loaded with 2 mL of a 0.25 M solution containing *p*-toluenesulfonic acid (*p*-TsA) as catalyst. Additionally, 350 mg of potassium bromide (KBr) were pre-added to enhance LevA yields, in accordance with Marcotullio and de Jong [30,31]. Experiments were performed by triplicate, at 190 ºC for 20 min, following the aforementioned studies [24,29].

After reaction, a dark crude product was observed. The mixture underwent filtration and thorough washing with deionized water. An orange liquid fraction was obtained and subsequently subjected to extraction with ethyl acetate (3×15 mL). The resulting organic (light yellow) and aqueous (orange) fractions were separately evaporated under vacuum, and the obtained dried crudes were analysed by NMR.

2.4. *Nuclear Magnetic Resonance (NMR) analysis*

^{1}H NMR issues were conducted on a Bruker Ascend™ 500 spectrometer (Bruker Corporation, Billerica, MA, USA) at 500.16 MHz for

the ^{1}H nucleus.

The organic crudes obtained in LevA transformation were analysed following the procedure previously described by Salgado-Ramos et al. [24] for quantitative purposes using quantitative NMR (qNMR). The acquisition parameters were: acquisition time: 2.723 min; scans number: 16; pulse: 90 ∘(8 μs); relaxation delay (D1): 5 s.

For aqueous crudes, 600 μL of deuterium oxide (D_2O) were used as the solvent. The sample preparation workup was similar to that for organics. In this case, NMR was used without quantification, therefore the parameters applied were: acquisition time: 1.19 min; number of scans: 16 scans; pulse: 90º (8 μs); relaxation delay (D1):1 s.

2.5. Statistical analysis

Statistical analysis was conducted on TAC and TPC results comparing soaking samples as the control against MAE using a multiple t-test with $p<0.05$. For LevA, the same analysis was applied, considering the experiments on the untreated matrix as the control ($p<0.05$). Results were expressed as mean ± standard deviation (SD), and processed using GraphPad Prism 8.0.2 software (GraphPad Software, San Diego, CA, USA).

3. Results and discussion

3.1. Recovery of bioactive compounds via microwave-assisted extraction (MAE)

MAE was applied to GS, GM and EGM to recover bioactive compounds (BACs), following the experimental conditions used in previous research for other lignocellulosic waste, such as almond hull biomass [24]. Total antioxidant capacity (TAC) and polyphenols content (TPC) were evaluated, and results summarised in Fig. 2. Conventional soaking was used as the control, for the sake of comparison.

At this point, and considering the methodology described in Section 2.3.1.1, the reason for increasing soaking time to 24 h compared to the 120 min used with MW is to mitigate potential variations in the physical state of the samples, which could introduce variability in the results of the extraction process. Thus, the dried and milled solid used in MW treatment may possess a higher surface area and more uniform particle size, facilitating a more efficient extraction compared to the raw, untreated solid used for soaking. This difference is addressed by prolonging the soaking time (24 h *vs.* 2 h with MW), assuming that the results obtained can be reasonably comparable. Additionally, soaking was carried out with the raw material on the basis of the methodology developed in previous studies [32].

As depicted in Fig. 2, higher TAC and TPC values were observed after MAE compared to soaking for EGM ($p<0.001$). However, this phenomenon was not observed for GS and GM, which is quite surprising. This discrepancy may be attributed to a significantly higher recovery of glycosylated polyphenols from these waste compared to the soaking treatment. Concretely, for GS and GM, the recovery of malvidin-3-glucoside or cyanidin-3-O-glucoside agrees with previously reported results in the literature [33,34]. The substitution of hydroxyl groups in the aromatic rings of polyphenols with sugar chains leads to a decrease in terms of scavenging activity, as these hydroxyl groups play a crucial role in the inhibition of free radicals [35,36].

For GM, the TEAC values after MAE (91.34 μmol TE/g DW) were significantly lower than those obtained after soaking (256.44 μmol TE/g DW) ($p<0.001$) (Fig. 2b). Similar trends were observed for ORAC, with values of 36.81 and 143.25 μmol TE/g DW for MAE and soaking, respectively ($p<0.0001$). Finally, the TPC values were also significantly different ($p<0.0001$), albeit with a narrower margin, standing at 11.98 and 53.35 mg GAE/g DW for MAE and soaking, respectively.

The recovery of antioxidants from GM via MAE has been extensively documented [33,34,37]. However, in this study, only moderate TAC and TPC were obtained. As a result, the outcomes for this particular residue were not subjected to an in-depth analysis.

Table 1
Phenolic concentrations by LC-MS for the microwave-assisted extracted (MAE)-crude obtained from exhausted grape marc (EGM).

Compound	Formula	Retention time (min)	Concentration (mg/Kg)
Quercetin 3-O-glucuronide	$C_{21}H_{18}O_{13}$	10.84	490.79
Quercetin	$C_{15}H_{10}O_7$	12.51	165.40
(+,-) catechin, epicatechin	$C_{15}H_{14}O_6$	6.32	130.12
Procyanidins dimer B1-B7	$C_{30}H_{26}O_{12}$	7.30	51.49
Nepetin	$C_{16}H_{12}O_7$	13.39	7.17
Syringic acid	$C_9H_{10}O_5$	9.31	6.79
Delphinidin 3,5-O-diglucoside	$C_{27}H_{31}O_{17}$	10.31	3.59

In the case of GS, once again, the impact of MAE was not as prominent as soaking. TEAC value after MW treatment was 148.24 μmol TE/g DW, significantly lower than the 1047.28 μmol TE/g DW obtained after soaking ($p<0.0001$) (Fig. 2a). Regarding ORAC, although the difference was less pronounced ($p<0.001$), values for MAE (132.07 μmol TE/g DW) were still markedly lower than those for soaking (416.25 μmol TE/g DW). Finally, TPC values also showed significant differences ($p<0.001$), with 52.22 and 108.67 mg GAE/g DW for MAE and soaking, respectively.

In contrast to GM, the recovery of BACs from GS via MAE has not been explored until date. Only a previous work with the same matrix reported a delignification step with NaOH and NaDESs for lignin exploitation, with the liquid fraction evaluated by DPPH assay [9]. Therefore, these results represent a significance advancement in the state-of-the- art for GS and lay the foundation for further exploration of this alternative valorisation route for this residue in future studies.

For EGM, both the antioxidant capacity and polyphenols content were remarkable (Fig. 2**c**). ORAC values after soaking were 218.07 μmol TE/g DW, witnessing a significant increase to 339.30 μmol TE/g DW after MAE ($p=0.0107$). TAC by TEAC was even more enhanced with this technique ($p=0.0002$), specifically 436% higher than the values detected after soaking (34.65 and 185.74 μmol TE/g DW for soaking and MAE, respectively). Finally, a 32% increase in TPC values was observed after MAE compared to soaking as well ($p=0.0024$), rising from 20.60 to 27.21 mg GAE/g DW.

Similarly to GS, no previous reports were found in the available literature for EGM after applying MAE, making it challenging to draw comparisons. In summary, MAE emerges as a promising approach to recover BACs from winemaking waste. While this study excludes conclusive results for GM, it marks the first application to GS and EGM, enhancing efficiency for the latter by up to 436% in terms of TAC and 32% by TPC.

Furthermore, the phenolic profile of the antioxidant-rich liquid extract obtained from EGM via MAE was determined (Table 1). Briefly, the presence of glycosylated structures, consistent with literature findings for EGM [27,38], was observed. Notably, quercetin 3-O-glucuronide or delphinidin 3,5-O-diglucoside were the main compounds (Table 1). Regarding non-glycosylated, flavonoids, including flavonols (quercetin), anthocyanins (procyanidins dimer B1-B7), flavanols (catechin, epicatechin) and flavones (nepetin) predominated. In parallel, hydroxybenzoic acids, such as syringic acid, were also detected.

Among the aforementioned compounds, for instance, quercetin has potential benefits in type 2 diabetes [39], flavones show promise in ovarian cancer [40], and flavanols exhibit anti-obesity capacity [41]. Noteworthy is the selective extraction of procyanidins dimer B1-B7 with MAE, in agreement with previous report on other matrices [24]. In summary, the phenolic profile reported for EGM in this study improves the current understanding, primarily due to the scarcity of literature. Nonetheless, further research is needed for a comprehensive

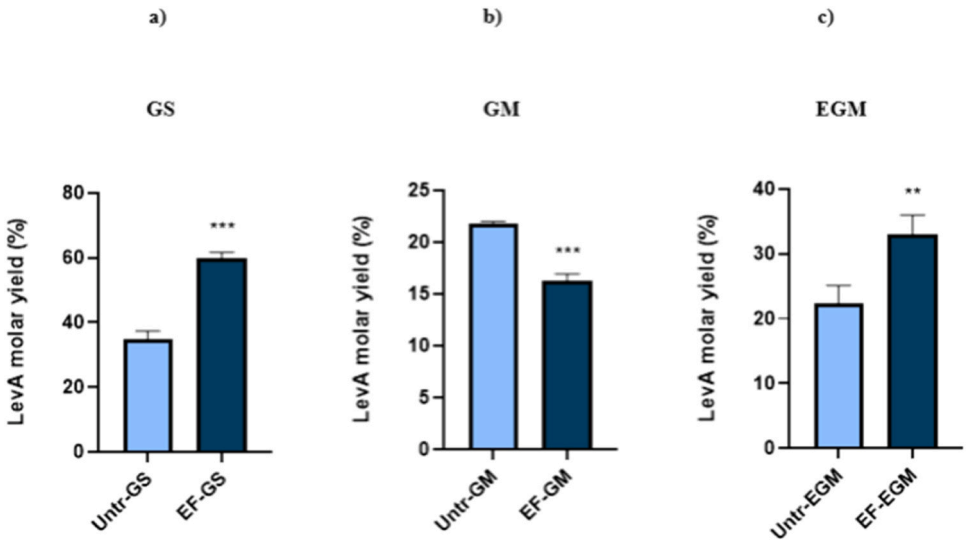

Fig. 3. Conversion of winemaking waste to levulinic acid (LevA) via microwave (MW)-assisted heating: grape stalks (GS) (a), grape marc (GM) (b) and exhausted grape marc (EGM) (c). Abbreviations: untr: untreated; EF: extractives-free. *** $p<0.001$; $p<0.01$ *vs.* control (untreated matrices).

understanding of this matrix and for comparison with other extensively studied winemaking waste.

3.2. Microwave (MW)-assisted conversion to levulinic acid

The conversion of GS, GM and EGM to levulinic acid (LevA) was carried out using a green processing approach with MW, following established studies [29]: temperature and time: 190ºC, 20 min; catalyst: *p*-toluenesulfonic acid (*p*-TsA) (acidic-aqueous solution), addition of KBr as co-catalyst, and without the use of an organic solvent. These conditions afforded a 50% molar yield of LevA from microcrystalline cellulose, which were subsequently confirmed in a real lignocellulosic material as melon rind (50% LevA molar yield) [29]. Thus, aiming to demonstrate the adaptability and feasibility of this developed approach for GS, GM and EGM materials, these optimised conditions were applied in this work, foregoing any further optimization efforts.

This reaction, originating from the cellulose fraction present in lignocellulosic biomass, typically results in the intermediate 5-hydroxymethylfurfural (5-HMF) (Figure S1). However, the use of an aqueous-single phase in these experiments allowed the continuation of the reaction until LevA (Figure S1), with no traces of 5-HMF observed in each transformation.

In this context, the use of *p*-TsA stands out compared to mineral acids for several reasons. Firstly, it possesses a similar pKa value (-2.8) to commonly used H_2SO_4 (-3). Additionally, it offers a cleaner process, preventing corrosion unlike these mineral acids. Finally, it can be easily recovered from the medium after the reaction (Figure S2), which highlights its environmentally friendly properties.

For these experiments, two types of matrices were considered: i) untreated (untr)-GS, GM and EGM, and ii) extractives-free (EF)-GS, GM and EGM, obtained after fibre characterisation (summarised in Table S1, according to previous work [32]). The removal of lipophilic and hydrophilic metabolites from the untreated samples makes the remaining lignocellulosic fraction more accessible in the EF matrix [32], which could lead to variability in the final LevA yield. Results for the conversion to LevA are detailed in Fig. 3.

As can be depicted from Fig. 3, the results for GS were remarkable compared to both GM and EGM. For instance, the LevA yield reached the maximum value when considering only the untreated matrix (34.75% *vs.* 21.83 and 22.31% for GM and EGM, respectively). The lower proportion of extractives in GS (Table S1) might facilitate the attack of the catalyst on cellulose, thus favouring the subsequent transformation. In fact, for EF-GS, the yield increased to 59.83% after removing these extractives (Fig. 3a), which supposes a significant improvement with respect to untr-GS ($p=0.0002$). Moreover, this cellulose fraction is typically enveloped by the lignin framework within lignocellulosic matrices. Therefore, the lower proportion of lignin in GS compared to GM and EGM (27.81% *vs.* 41.32 and 44.91%, respectively) (Table S1) could also explain these results.

To date, only one report in the literature detailed the production of LevA from GS, using a cascade protocol for full grape stalk valorisation [10]. This study involves a preliminary delignification step by employing a combination of ultrasound-assisted extraction (UAE) with natural deep eutectic solvents (NaDESs), concretely choline chloride (ChCl) + LevA (ChLevA). Subsequently, the remaining solid fraction underwent a transformation into LevA assisted by MW radiation, with the untreated GS used as sake of comparison. Remarkably, molar yields of 85.1 and 68.6% were respectively achieved. The reaction conditions were adapted from the work of Tabasso et *al.* 2014 [42], and included a temperature of 225ºC, a duration of 2 min, HCl 1 M as catalyst, and a pressure of 40 bar with nitrogen (N_2) atmosphere.

In contrast, this study developed a greener process, achieving considerable LevA yield (59.83%). Notably, the use of a reusable catalyst, as *p*-TsA, and lower temperatures contributed to a more energy-efficient transformation.

Further, considering EGM (Fig. 3c), the significant increase in LevA yield (33.09% from EF and 22.31% from the untreated matrix, $p<0.01$) may relate to the recovery of lipophilic and hydrophilic compounds. For GM, by contrast, no noticeable results were obtained (Fig. 3b). In fact, as the opposite as occurred with both GS and EGM, LevA significantly decreased ($p<0.001$) for EF-GM (16.80%) compared to the untreated matrix (21.83%). This discrepancy highlights the changeable results depending on the initial feedstock, emphasising the complexity of lignocellulosic materials. Nonetheless, this study marks the first development of LevA from GM and EGM, showcasing a distinguished progress for GS in terms of environmental sustainability with respect to the available literature. In summary, these results pave the way to widen the existing research in this regard, mainly considering the suitability of these matrices towards LevA.

Simultaneously, the removal of primary metabolites was monitored by ^{1}H NMR. Thus, the liquid crude obtained after extraction was characterized (Figure S3). For illustrative purposes, the NMR spectra of the lipophilic and hydrophilic fraction obtained from EGM are attached. For the latter, mainly freely accessible carbohydrates were detected

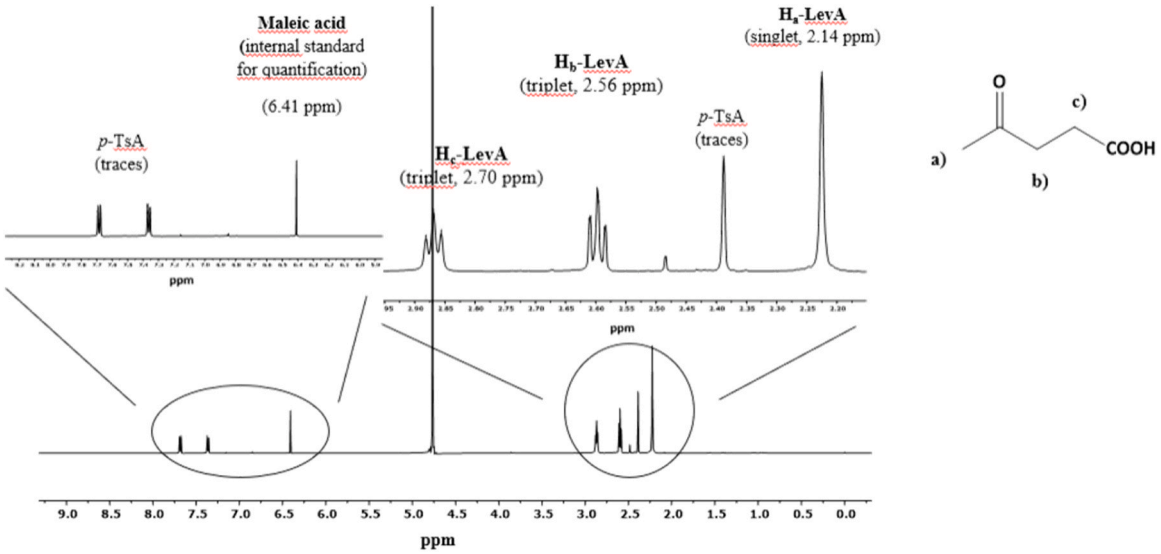

Fig. 4. ^{1}H NMR spectrum (D_2O, 500 MHz) of the organic crudes obtained in the transformation of winemaking waste into levulinic acid (LevA) via microwave (MW) heating.

(Figure S3b), ranging from 5.5 to 3 ppm, whereas the presence of lipidic compounds (acyl glycerides) was remarkable for the former (Figure S3a). As mentioned, the recovery of these compounds was crucial to enhance LevA yield in GS and EGM. Moreover, this fraction could be concurrently exploited for the development of other high-added-value chemicals, as previously reported [24,26].

Finally, it should be noted that LevA was selectively obtained *vs*. 5-HMF in each transformation, as observed by the ^{1}H NMR analysis of the organic crudes recovered after reaction (Fig. 4). Moreover, quantitative conversions from glucose were achieved, as no residual glucose was detected in the aqueous phase (see Figure S2, region between 5.5 and 3 ppm (freely accessible carbohydrates)).

4. Conclusions

In accordance with process intensification, sustainable development and circular economy principles, this study has comprehensively advanced a novel approach for upgrading for grape stalk (GS), grape marc (GM) and exhausted grape marc (EGM) via MW radiation in a two-way process.

Firstly, the recovery efficiency of bioactive compounds (BACs) from EGM witnessed an unprecedent surge, achieving an impressive 436% increase in total antioxidant capacity compared to the traditional soaking methods. This enhancement, coupled with the identification of valuable polyphenols such as flavonols (quercetin), flavanols (catequin, epicatechin) or anthocyanins (procyanidins (B1-B7) through LC-MS analysis, underscores the immense potential of EGM as a rich source of bioactive compounds.

Simultaneously, an environmentally sustainable strategy was employed for LevA production, emphasising a single-phase-based system with water (H_2O), reusable catalysts such as *p*-TsA, and short reaction times (20 min). The transformations entailed total conversion and selectivity to LevA, with remarkable yield for GS matrices (34.73 and 59.83% from untreated- and EF-GS, respectively), which demonstrated the feasibility and efficiency of the proposed approach. This study not only conforms to the core principles of Green Chemistry, but also represents a pivotal stride towards establishing sustainable biorefineries derived from winemaking practises. Additionally, the comprehensive investigations yielded significant findings that hold substantial implications for advancing the valorisation of these materials, effectively addressing the research gap particularly in terms of LevA production, identified in the literature on this subject.

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CRediT authorship contribution statement

Manuel Salgado Ramos: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **M. Prado Sánchez-Verdú:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Francisco J. Barba:** Writing – review & editing, Validation, Supervision, Conceptualization. **Francisco J. Martí-Quijal:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Alberto J. Huertas-Alonso:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Beatriz Cabañas:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Andrés Moreno:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

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

Data Availability

No data was used for the research described in the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2024.112411.

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