

Production of levulinic acid and *n*-butyl levulinate from the waste biomasses grape pomace and *Cynara Cardunculus* L.[†]

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Abstract: Nowadays, the transformation of lignocellulosic biomass into added-value products, such as bio-based platform chemicals and fuels, is attracting great attention. In this research, the acid-catalyzed hydrolysis of waste grape pomace and *Cynara Cardunculus* L. (cardoon), remained after wine and oil exploitation respectively, to levulinic acid was investigated. The grape pomace residue was employed as received, whereas the cardoon one was used both as received and after the steam explosion treatment, becoming enriched in cellulose. Since waste biomasses are low value materials, this investigation was performed adopting the *High Gravity* approach, using biomass loadings as higher as possible in order to achieve the maximum product concentration in the hydrolyzates, while maintaining an appreciable yield. Under the optimized reaction conditions, starting from grape pomace residue, the levulinic acid yield and concentration of 49.5 mol% and 8.5 g/L were reached respectively, whereas, from pre-treated cardoon residue, levulinic acid yields and concentrations up to 53 mol% and 62 g/L were attained, highlighting cardoon as the most suitable feedstock. On this basis, one-pot alcoholysis of pre-treated cardoon in *n*-butanol to *n*-butyl levulinate was successfully performed. In summary, waste cardoon resulted a promising feedstock for the sustainable production of both levulinic acid and alkyl levulinates, paving the way towards a circular economy.

Keywords: levulinic acid; *n*-butyl levulinate, grape pomace, *Cynara Cardunculus* L., *High Gravity* approach

1. Introduction

Renewable resources have garnered increasing interest due to shortage of petroleum, uncertainty of its price and environmental deterioration associated with its consumption. The serious need to explore alternative resources to traditional ones for the production of chemicals and fuels has encouraged a new international policy of energy, including the demand of increasing amount of renewable fraction inside bio-fuels. In this scenario, chemical conversion of nonedible biomass to bio-molecules suitable for new applications should be developed and implemented, without any conflict with food chain. In particular, the ability to employ as starting material residual or waste biomasses is an added value and fits with the concept of circular economy. In this regard,

the hydrolysis of lignocellulosic biomass is an efficient way to produce valuable platform chemicals, such as levulinic acid (LA) [1]. Due to multiple functional groups, including one carboxyl and one carbonyl, LA can be converted in various products, such as bio-fuels and intermediates for chemical and pharmaceutical industries [2]. In particular, some of the most promising LA derivatives for biofuels production are alkyl levulinates (ALs) that can be advantageously used as cold flow improvers in biodiesel and as oxygenate additives for gasoline and diesel fuels [2]. However, the yield of LA synthesis is strongly affected by the type of feedstock [1, 3]. Indeed, biomass can be made of variable content of polymers, such as cellulose (30-55 wt%), hemicellulose (20-50 wt%) and lignin (10-30 wt%) depending on its species and origin and the composition can change by applying pre-treatments [3]. Since LA is mainly produced via hydrolysis of hexose sugars, such as glucose, cellulose-rich biomasses result the most suitable feedstock [1]. On this subject, *Cynara Cardunculus* L. (cardoon) represents a potential candidate as starting material for LA green synthesis: it is a versatile Mediterranean crop with a wide spectrum of potential applications, being a rich source of fibers, oils and bioactive compounds. Profitably, the cultivation of such perennial herbaceous is adapted to climate change and it can easily grow on marginal or uncultivated lands with modest inputs, such as little irrigation, care and minimal need of nutrients [4]. The seeds of the flower are recovered for oil production and employed in food-chain or biodiesel supply chain. On the other hand, the nonedible lignocellulosic residues of this crop can undergo pre-treatments that favor further exploitation of this biomass [5]. Steam explosion is the most commonly used and cost-effective method for pre-treatment of lignocellulosic materials [6]. This procedure causes hemicellulose degradation and lignin transformation due to high employed temperatures, and, in addition, it reduces the crystallinity of cellulose, thus increasing its accessibility to hydrolysis.

In the present research, two types of cardoon waste residues, remaining after seeds removal, were studied: the first one is the non-treated material, whereas the second one is the waste residue after the steam-explosion pre-treatment. Acid-catalyzed hydrolysis, assisted by microwave (MW) irradiation, was successfully performed for both samples. Taking into account the high reached LA concentration, the hydrolysis of steam-exploded cardoon was also performed in batch autoclave in the perspective of the industrial scale-up. In addition to this biomass, also grape pomace, the major solid waste resulting from the wine industry and consisting in skins, seeds and stems remained after grape fruit processing, was investigated. Grape pomace is mainly as a source of polyphenols for cosmetic applications [7] but interestingly, it has recently been investigated also for the production of monosaccharides and activated carbons, due to its content in carbohydrates (about 10-35 wt%) and lignin (about 10-30 wt%), respectively [8]. However, its cellulose fraction, albeit low (5-20% wt), could be further exploited after polyphenols recovery, in particular for the production of LA developing an integrated process.

Finally, taking into account that the pre-treated cardoon resulted the most suitable feedstock, the direct production of levulinates by alcoholysis of this residues was also studied, resulting this strategy more promising than the generally adopted two-steps procedure consisting of *i*) synthesis of LA and *ii*) its esterification. [2]. Indeed, the one-pot synthesis does not require intermediate operations concerning LA concentration and purification and reduces also the waste water treatment [9]. In particular, the one-pot alcoholysis was performed in MW reactor in *n*-butanol to produce *n*-butyl levulinate (BL). The highly interesting results of both hydrolysis and butanolysis highlight cardoon as a promising feedstock for multi-products biorefineries.

2. Methods

2.1. Materials

Two types of cardoon residues were investigated: non pre-treated cardoon (C) and steam explosion pre-treated one (E). Also non pre-treated red grape pomace residues (R) was studied. Catalysts and chemicals were purchased from Sigma-Aldrich and employed as received (see ref. [9,10]).

2.2. Acid-catalyzed hydrolysis of cardoon and grape pomace

Hydrolysis reactions were carried out in deionized water, using HCl as catalyst, in the single-mode MW reactor CEM Discover S-class System at 190°C for 20 minutes. Batch experiments were carried out also in an electrically heated 600 mL Parr zirconium made-fixed head autoclave, equipped with a P.I.D. controller (4848). The reactions in batch autoclave, pressurized with nitrogen up to 30 bar, were performed at the fixed temperature of 190°C for 2 hours. At the end of each reaction, the reactors were rapidly cooled at room temperature by blown air, the hydrolyze was separated from solid residuals by vacuum filtration, filtered with a PTFE filter (0.2 µm) and analyzed by means of high-pressure liquid chromatography with a refractive index detector, as reported in ref. [10]. The molar yield of each compounds of interest was calculated as (moles of product/moles of anhydrous glucose unit in starting biomass) × 100, while the ponderal yield as (grams of product/grams of dry starting biomass) × 100.

2.3. Acid-catalyzed alcoholysis of cardoon

Alcoholysis reactions were carried out in *n*-butanol, using H₂SO₄ as catalyst, in the MW reactor CEM Discover S-class System at 190°C for 15 minutes. At the end of each reaction, the slurry was filtered under vacuum on a crucible and the liquid samples were diluted with acetone and analyzed by a gas chromatograph coupled with a flame ionization detector, as reported in ref. [9].

3. Results and Discussion

3.1. Compositional analysis of waste residues

Figure 1 reports the chemical compositions of the investigated biomasses determined according to NREL protocol [12]. Both C and E have a high content of cellulose, about 38 and 65 wt%, respectively. However, after the steam explosion pre-treatment, hemicellulose amount decreases from about 17 to 4 wt%, and extractives and ash are removed. On the other hand, lignin increases from about 17 up to 30 wt%, mainly because of the reduction of the other components. Due to the high pressure wet pre-treatment, the resulting residue E shows a much higher content of humidity than C, whose values are about 73.6 and 5.9 wt%, respectively. As regards R, a modest fraction of cellulose, 13.9 wt%, and a significant content of lignin, 47.6 wt%, were determined, while the content of humidity resulted of 66.0 wt%.

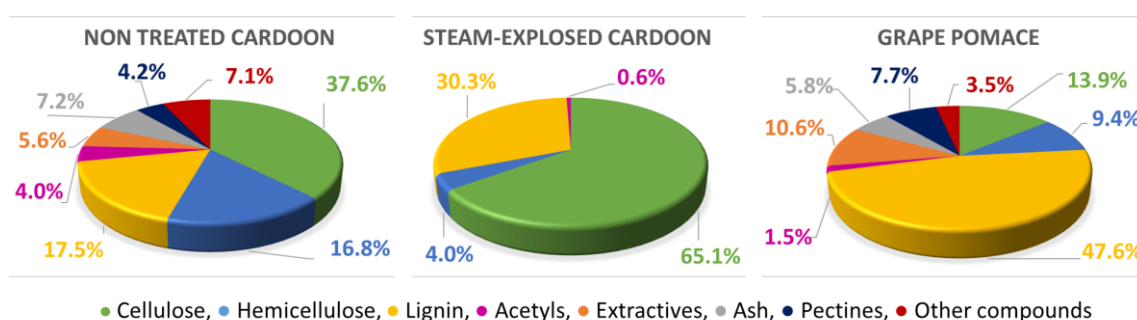


Figure 1. Chemical compositions of the investigated biomasses reported as wt% over dry biomass.

3.2. Hydrolysis of C and E cardoon

In the preliminary study, both wet and dry samples of C and E cardoon residues were investigated (See Table S1 in Supplementary Materials). No moisture effect was observed on the formation of LA nor on that of formic acid (FA), which is another commodity chemical co-produced during biomass hydrolysis [2]. For this reason, only wet samples of both C and E residues were considered for the more detailed investigation. The *High Gravity* approach was used, increasing the biomass loading as much as possible in order to achieve the highest products concentration, making

easier the purification step. Table 1 (runs C1-C3 and E1-E3) reports the results of hydrolysis experiments with progressively increased biomass loading (from 10 up to 20 wt%). No important decrease of LA yields was observed but higher LA concentrations, from 15.9 up to 34.6 g/L for C samples and from 27.8 to 59.7 g/L for E ones were reached.

Table 1. Results of the hydrolysis experiments on C and E cardoon (190°C, HCl as catalyst).

Run	Reactor	Biomass loading (wt%)	Sub/cat (mol/mol)	Time (min)	LA (g/L)	LA PY (wt%)	LA MY (mol%)	FA (g/L)
C1	MW	10	0.9	20	15.9	13.6	50.7	8.5
C2	MW	15	0.9	20	23.9	13.0	48.1	12.7
C3	MW	20	0.9	20	34.6	13.0	48.5	12.7
C4	MW	20	1.5	20	27.8	10.7	39.8	13.4
C5	MW	20	2.0	20	34.3	13.3	49.6	12.7
E1	MW	10	0.9	20	27.8	23.6	49.7	9.3
E2	MW	15	0.9	20	47.0	24.6	51.8	11.7
E3	MW	20	0.9	20	59.7	21.1	44.5	12.5
E4	MW	20	1.5	20	55.4	20.7	43.6	15.9
E5	MW	20	2.0	20	59.0	22.4	47.3	20.4
E6	MW	20	2.0	40	62.1	23.6	49.9	19.3
E7	MW	20	2.0	60	57.5	21.8	46.1	12.9
E8	Autoclave	10	0.9	120	29.3	25.2	53.1	10.5

Homogenous catalysts are preferable when solid substrate is employed and among them, HCl is a powerful agent for cellulose degradation, although it is hazardous and requires corrosion-resistant and expensive reactors. Therefore, in order to limit this problem, it is of paramount importance to use low concentration of HCl. In this regard, Table 1 (runs C3-C5 and E3-E5) reports the results of experiments performed with different HCl amounts, varying the substrate/catalyst ratios (sub/cat) from 0.9 up to 2.0 mol/mol at 20 wt% of biomass. It is interesting to highlight that LA molar yield is not affected by the reduction of catalyst content, at least in the investigated range of sub/cat. In general, for both E and C samples, promising values of LA molar yields are obtained and no significant amounts of side-products, such as non-converted glucose, HMF, furfural and acetic acid, are observed. However, higher LA concentrations are achieved from steam-exploded cardoon than the non-treated one. On the basis of these results, treated cardoon E was selected for further studies regarding the effect of reaction time on LA formation. As reported in Table 1 (runs E5-E7), experiments were carried out at different reaction times, from 20 to 60 minutes, at the lowest amount of catalyst. A slight time effect was observed on the hydrolysis efficiency, achieving the best LA yield of about 50 mol% together with the highest LA concentration of 62.1 g/L, both after 40 minutes of heating. In other words, green and energy saving conditions, such as low acid concentration and shortened heating, for the conversion in water of significant amount of E biomass can be adopted.

Hydrolysis reaction of E sample was also performed in batch autoclave in order to adopt operational conditions more similar to those of industrial plants. Longer reaction times, 120 minutes, were applied in autoclave, due to its slow transient heating. In the case of biomass loading of 10 wt%, similar LA molar yields were observed in MW and autoclave, about 50 and 53 mol% (runs E1 and E8), respectively, demonstrating that both heating methodologies can be successfully applied for the biomass conversion process.

3.3. Hydrolysis of R grape pomace

On the basis of a screening test on different types of grape pomace (Table S2 in Supplementary Materials), red residue (R) resulted the most reactive and was employed in MW-assisted hydrolysis experiments, whose results are reported in Table 2. At the beginning, employing 15 wt% of biomass

loading and 20 minutes of reaction time (run R1), an appreciable amount of non-converted glucose was detected. On this basis, the reaction time (R2) and catalyst amount (R3) were increased in order to obtain a more efficient sugar conversion. However, the concentration of LA still remained low (8-9 g/L), even with increased biomass loading (20 wt% in run R4), due to the modest initial content of cellulose. These results are not comparable with the ones obtained employing E cardoon, which resulted the most suitable feedstock for LA production.

Table 2. Results of the hydrolysis experiments on R grape pomace (MW heating, 190°C, HCl as catalyst).

Run	Biomass loading (wt%)	Sub/cat (mol/mol)	Time (min)	LA (g/L)	LA PY (wt%)	LA MY (mol%)	FA (g/L)	Glucose (g/L)
R1	15	0.3	20	8.1	4.4	47.0	5.0	8.9
R2	15	0.3	40	8.5	4.6	49.5	6.1	-
R3	15	0.1	20	9.2	4.7	50.8	5.4	-
R4	20	0.3	40	8.2	3.1	33.6	5.5	-

3.4. Alcoholysis of *E cardoon*

In the second part of our investigation, the one-pot alcoholysis reaction of *E cardoon* in *n*-butanol to produce BL was carried out in MW reactor. In presence of a low content of acid catalyst (1.2 wt% of H₂SO₄) and applying only 15 minutes of heating, the butanolysis led to the BL molar yield of about 43 mol% using the biomass loading of 8 wt% (Table 3). In addition to BL, di-butyl-ether (BE) which is another valuable compound for biofuels [13] was formed from butanol dehydration. This is a preliminary result but it demonstrates that cardoon is a promising feedstock also for the direct BL production.

Table 3. Results of the butanolysis experiment on *E cardoon* (MW heating, 190°C, H₂SO₄ as catalyst).

Run	Biomass loading (wt%) ¹	Catalyst loading (wt%)	Time (min)	BL (g/100 g) ²	BE (g/100 g) ²	BL PY (wt%)	BL MY (mol%)
BL1	8	1.2	15	30.00	81.69	30.0	42.5

¹The reported biomass loadings are referred to dry biomass.

²The reported products amounts are expressed as grams of product for 100 g of starting dry biomass.

4. Conclusions

In this paper, the acid-catalyzed hydrolysis of waste cardoon and grape pomace residues from agro-industry to the valuable platform chemical LA was investigated. Non-treated biomasses were employed but in the case of cardoon, also steam-exploded residue, enriched in cellulose, was studied. Hydrolysis reactions of these biomasses carried out in MW reactor and/or in autoclave led to high values of LA molar yield and concentration, up to about 53 mol% and 62 g/L, respectively, for steam-exploded cardoon. In this regard, the reaction performed in autoclave demonstrates as such process can be realized in traditional industrial reactors. Among grape pomace, the red residue (R) resulted the most reactive, achieving the LA molar yield and concentrations of about 50 mol% and 10 g/L, respectively. Finally, a preliminary test on acid-catalyzed butanolysis of steam-exploded cardoon, with the appreciable BL yield of about 43 mol%, proves that innovative processing plants fed by such waste biomass can supply a wide range of value-added products, including bio-fuel additives. In conclusion, the proposed approaches represent an alternative solution to reduce consumption of fossil resources and to recycle massive amounts of agricultural residues in line with the concept of third generation biorefinery.

Supplementary Materials: The following are available online at <https://sciforum.net/conference/ECCS2020>, Table S1: preliminary study on biomass humidity effect on C and E cardoon.; Table S2: Preliminary study on different types of grape pomace: yellow residue (Y) and red residue (R).

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References

1. Antonetti, C.; Licursi, D.; Fulignati, S.; Valentini, G.; Raspolli Galletti, A.M. New Frontiers in the Catalytic Synthesis of Levulinic Acid: From Sugars to Raw and Waste Biomass as Starting Feedstock. *Catalysts* **2016**, *6*, 196–225. Doi: 10.3390/catal6120196.
2. Leal Silva, J.F.; Grekin, R.; Pinto Mariano, A.; Maciel Filho, R. Making levulinic acid and ethyl levulinate economically viable: a worldwide techno-economic and environmental assessment of possible routes. *Energy Technol.* **2018**, *6*, 613–639. Doi: 10.1002/ente.201700594.
3. Rackemann, D.W.; Doherty, W.O. The conversion of lignocellulosics to levulinic acid. *Biofuels, Bioprod. Bioref.* **2011**, *5*, 198–214. Doi: 10.1002/bbb.267.
4. Grammelis, P.; Malliopoulou, A.; Basina, P.; Danalatos, N.G. Cultivation and Characterization of *Cynara Cardunculus* for Solid Biofuels Production in the Mediterranean Region. *Int. J. Mol. Sci.* **2008**, *9* (7), 1241–1258. Doi: 10.3390/ijms9071241.
5. Fernández, J.; Curt, M.D.; Aguado, P.L. Industrial applications of *Cynara Cardunculus* L. for energy and other uses, *Ind. Crops Prod.* **2006**, *24* (3), 222–229. Doi: 10.1016/j.indcrop.2006.06.010.
6. Overend, R.P.; Chornet, E.; Gascoigne, J. Fractionation of lignocellulosics by steam-aqueous pretreatments. *Philos. T. R. Soc. A* **1987**, *321* (1561) 523–536. Doi: 10.1098/rsta.1987.0029.
7. Álvarez, A.; Poejo, J.; Matias, A. A.; Duarte, C. M. M.; Cocero, M. J.; Mato, R.B. Microwave pretreatment to improve extraction efficiency and polyphenol extract richness from grape pomace. Effect on antioxidant bioactivity. *Food Bioprod. Process.* **2017**, *106*, 162–170. Doi: 10.1016/j.fbp.2017.09.007.
8. Pedras, P.; Salema-Oom, M.; Sá-Nogueira, I.; Simões, P.; Paiva, A.; Barreiros, S. Valorization of white wine grape pomace through application of subcritical water: Analysis of extraction, hydrolysis, and biological activity of the extracts obtained. *J. Supercrit. Fluids* **2017**, *128*, 138–144. Doi: 10.1016/j.supflu.2017.05.020.
9. Antonetti, C.; Gori, S.; Licursi, D.; Pasini, G.; Frigo, S.; López, M.; Parajó, J. C.; Raspolli Galletti, A. M. One-Pot alcoholysis of the lignocellulosic *Eucalyptus nitens* biomass to *n*-butyl levulinate, a valuable additive for diesel motor fuel. *Catalysts* **2020**, *10*, 509–530. Doi: 10.3390/catal10050509.
10. Rivas, S.; Raspolli Galletti, A.M.; Antonetti, C.; Santos, V.; Parajó, J.C. Sustainable conversion of Pinus pinaster wood into biofuel precursors: a biorefinery approach. *Fuel* **2016**, *164*, 51–58. Doi: 10.1016/j.fuel.2015.09.085.
11. a) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. Determination of Structural Carbohydrates and Lignin in Biomass. Laboratory Analytical Procedure (LAP), NREL/TP-510-42618, **2008**; b) Sluiter, A.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. Determination of Extractives in Biomass. Laboratory Analytical Procedure (LAP), NREL/TP-510-42619, **2008**; c) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. Determination of Ash in Biomass. Laboratory Analytical Procedure (LAP), NREL/TP-510-42622, **2008**.
12. Koivisto E.; Ladommatos N.; Gold M. The influence of various oxygenated functional groups in carbonyl and ether compounds on compression ignition and exhaust gas emission. *Fuel* **2015**, *159*, 697–711.



Table S1. Preliminary study on biomass humidity effect on C and E cardoon.

Run ^a	Reactor	Biomass loading (wt%)	Sub/cat (mol/mol)	Time (min)	LA (g/L)	LA PY (wt%)	LA MY (mol%)	FA (g/L)
C wet	MW	10	0.9	20	15.9	13.6	50.7	8.5
C dry	MW	10	0.9	20	23.9	13.0	48.1	12.7
E wet	MW	10	0.9	20	27.5	23.6	49.7	9.3
E dry	MW	10	0.9	20	25.5	22.0	46.2	9.4

^a 190°C, HCl as catalyst.

Table S2. Preliminary study on different types of grape pomace: yellow residue (Y) and red residue (R).

Run ^a	Biomass loading (wt%)	Sub/cat (mol/mol)	Time (min)	LA (g/L)	LA PY (wt%)	LA MY (mol%)	FA (g/L)	Glucose (g/L)
R1	15	0.3	20	8.1	4.4	47.0	5.0	8.9
Y1 ^b	15	0.3	20	6.3	3.3	34.1	6.6	5.8

^a MW heating, 190°C, HCl as catalyst.

^b Chemical composition reported as wt% over dry biomass: cellulose = 13.8%, hemicellulose = 8.4%, acetyls = 1.2%, lignin = 48.7%, extractives = 3.9%, pectines = 8.1%, ash = 6.3%, other compounds = 9.6%. Humidity content = 47.3%.