# Bioethanol Production from Globe Artichoke Residues: from the Field to the Fermenter

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## Abstract

The suitability of globe artichoke crop residues to be transformed into bioethanol was assessed in this paper from the field to the fermenter. A 2-year field trial on "Opera F1" (OF1), a hybrid variety of globe artichoke, resulted in an average annual production of residues of 14 t/ha. The residual biomass of OF1 is made up of 24% glucan and 17% hemicelluloses and is rich in soluble sugars. Water extraction (WE) (100 °C, 15 min) was initially applied to remove simple sugars that can be transformed into saccharification and fermentation inhibitors during the pretreatments. Subsequent dilute acid hydrolysis (DAH) ($H_2SO_4$ 2%, 121 °C, 1 h) produced a slurry with 17.7, 4.2, and 0.5% (dry matter: DM) of soluble sugars, acetic acid, and total furanic compounds, respectively, and a solid fraction with 52% glucan and 14% xylan, with practically 100% digestibility. Slurry enzymatic hydrolysis (45 FPU/g DM, 7 CBU/g DM, 24 h) had a 69% glucan yield, showing an inhibition of the saccharification process due to the presence of simple sugars and inhibitors. Co-fermentation of the enzymatic slurry with *Escherichia coli* MS04 produced 12.5 g/L ethanol with a volumetric productivity of 0.52 g/L/h and 76.0% fermentation efficiency after 24 h of fermentation. Considering all sugars generated during DAH, the applied strategy allowed a production of 283 kg/t DM and 2399 kg/ha of bioethanol, against the theoretical value of 2806 kg/ha.

**Keywords** Crop residues • *Cynara cardunculus* • Bioethanol • *Escherichia coli* MS04 • Dilute acid hydrolysis • Bioenergy

## Introduction

In the current search for new renewable sources of energy, lignocellulosic biomass (LCB) plays an important role. In this regard, one of the possible sources of LCB in the Mediterranean environment is represented by the crop residues of the globe artichoke (*Cynara cardunculus* L. var. *scoly- mus* (L.) Fiori), which is an herbaceous plant native to the Mediterranean basin and belongs to the Asteraceae family. It is traditionally grown in Mediterranean countries, as it is well adapted to its soil and climatic conditions, but is rapidly spreading to other countries, such as Peru, Argentina, China, and the USA [1]. Approximately one-third of the world globe artichoke production is made in Italy, where there are more than 43,000 ha of growing areas and ~ 413,000 t/ year are harvested (average for the decade 2010-2019) [1]. Numerous crops intended for human consumption leave a large amount of biomass, such as crop residues, on the ground after harvesting, and among these, globe artichoke is one of the most interesting in the Mediterranean environment. This is largely because of its growth cycle—lasting from early autumn to late spring—which avoids the hottest and driest time of the year [2]. There is little doubt that the ongoing climate change is one of the results of human activities [3] and, in particular, of the use of fossil fuels that emit carbon dioxide into the atmosphere that has been definitively removed from the carbon cycle. As sensitivity to the issue of climate change grows, interest in alternative energy sources is also on the rise, such as bioethanol from agricultural biomass. The production of bioethanol is still based on the use of plants that could be used for human food [4], while hunger and malnutrition are widespread throughout the world. Globe artichoke field residues are a non-food biomass, and their use as feedstock for the production of ethanol would not lead to competition between the food and agro-energy sectors for the use of land resources. Furthermore, globe artichoke cultivation is generally concentrated in productive districts, as happens in Spain, France, Egypt, and Italy, which would reduce transport costs from the field to the processing plant. In particular, globe artichoke cultivation
in Sicily (southern Italy) is concentrated in 5-6 productive districts; this means that large amounts of biomass would be available within a few kilometers.

The production of bioethanol from lignocellulosic biomass is obtained through the fermentation of hexoses and pentoses derived from the depolymerization of cellulose and hemicellulose, respectively. However, since the latter are recalcitrant to enzymatic hydrolysis, it is therefore necessary to subject these biomasses to pretreatments [5]. Dilute acid hydrolysis (DAH) is a pretreatment technique in which mineral acids at low concentrations, in combination with high temperatures, are used to hydrolyze hemicelluloses and make cellulose more accessible [6, 7]. The main advantages of DAH are its low cost and effectiveness in the hydrolysis of hemicellulose, while its main drawback is the production of fermentation inhibitors [7]. Compared to pretreatments with concentrated acids, DAH involves less acid waste but also higher temperatures. Alkaline hydrolysis can be performed at lower temperatures and pressures than DAH, with less sugar degradation, but takes longer time pretreatments [6]. Hydrothermal pretreatments are less polluting than DAH but require very high temperatures and pressures [7]. DAH has already been applied to the biomass of cultivated cardoon, and this work takes into account other authors' experiences [8-10]. More generally, *C. cardunculus* has been studied as an energy plant [11-17]. The oil obtained from its achenes can be used to produce biodiesel [18-20]. The suitability of *C. cardunculus* biomass to direct combustion or pyrolysis has already been studied [21-25], together with its capacity

to produce biomethane [26-29] and bioethanol [7, 30, 31]. This work is the continuation and extension of a previous study by Pesce et al. [32] and aims to deepen the knowledge of the globe artichoke post-harvest biomass as a feedstock to produce bioethanol. The crop residues of a hybrid variety propagated by achene (improperly called "seed") were subjected to water extraction (from here on WE) and then to a DAH. The resulting slurry underwent saccharification with subsequent fermentation of both pentoses and hexoses (cofermentation) by *Escherichia coli* MS04. To our knowledge, this is the first time a study has been carried out on *C. car- dunculus* over such a long path, from the field to the fermenter. Furthermore, this is the first time a co-fermentation of the total slurry from DAH has been applied to *Cynara* spp., without any detoxification step. Finally, the results are expressed in such a way as to provide an indication of how much bioethanol can be obtained from one ton of dry biomass and from one hectare of crop residues.

## Materials and Methods

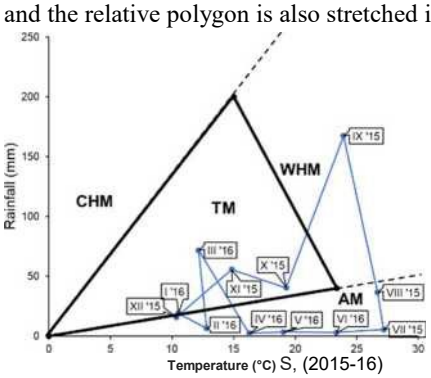
### Field Experiment

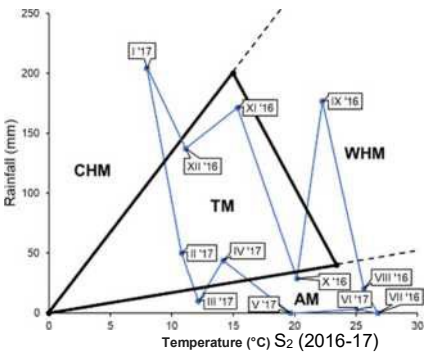
#### Climate and Soil

The field trial reported in this paper was conducted over two seasons, namely, 2015-2016 ($S_j$) and 2016-2017 ($S_2$), in the countryside around Siracusa, very close to the sea (37°03'N, 15°18'E, 10 m a. s. l.). Here, vegetables, such as potato, tomato, and aubergine, are traditionally cultivated, and globe artichoke is beginning to be grown. According to the Koppen climate classification, the site climate is typically Mediterranean, characterized by mild, rainy autumns and winters, and dry, hot summers. According to the Thornthwaite climatic classification, the climate of the site is defined as semiarid, tending to dry-subhumid. The monthly average maximum temperatures range from 15.7 (January) to 34.4 °C (August), while the monthly average minimum temperatures range from 4.6 (February) to 19.6 °C (August). The mean annual rainfall is 703 mm [33]. The soil is classified as a Calcixerollic Xerochrepts [34] and has the following texture: clay 18.5%, silt 26.1%, and sand 55.4%.

#### Meteorological Conditions During the Crop Cycle

The climograms of Peguy in Fig. 1 summarize the thermo- pluviometric conditions of the two seasons, which are compared with the long-term trend (1991-2020). The polygon that describes the long-term trend is rather elongated in the direction of the horizontal axis and represents a climatic condition characterized by high annual temperature ranges. Observing the climograms relating to $S_1$ and $S_2$, irregular trends clearly emerge. $S_1$ was particularly dry, with December '15 and January '16 bordering in arid months, while February '16 was decidedly dry. $S_2$, on the other hand, was wetter, with almost twice the rainfall compared to that of the first year, and the relative polygon is also stretched in the



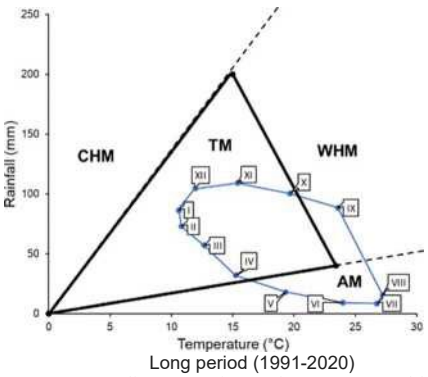



**Fig. 1** Peguy diagrams of $S_1$ and $S_2$ mean monthly temperature and monthly precipitation compared to the long-term average (1991-2020) in Siracusa. CHM cold and humid months, TM temperate months, WHM warm and humid months, AM arid months

direction of the vertical axis (Fig. 1). While the temperatures recorded in $S_1$ are roughly comparable to those of the long term, $S_2$ was characterized by low temperatures. The average of its mean monthly temperatures is in fact approximately half a degree Celsius lower than that of the long term (17.6 vs. 18.1 °C) [33].

### Plant Material, Crop Management, and Plant Sampling

"Opera F1" (hereafter OF1), an early purple F1 hybrid specifically bred for annual production, was studied. Its mode of propagation was by "seed." OF1 seedlings were transplanted in the last decade of August in a field previously plowed to a depth of ~ 30 cm. The distance between the plants in the row was 0.8 m, while the distance between the rows was 1.25 m, with a planting density of 1 plant per $m^2$. For fertilization, 150 kg/ha of N and 80 kg/ha of $P_2O_5$ were provided. The irrigation method was drip irrigation, applied when the daily evaporation reached 35 mm. The 100% of maximum evapotranspiration (ETm) was supplied. All inputs related to weed and pest control were carried out following standard practices and depending on the needs of the crop. A block consisting of 3 plots, each containing 100 plants, was identified in the middle of the field. Samples of globe artichoke field residues were collected from each plot. Field residues, consisting of stalks, leaves, and unharvested capitula, were collected in the central area (10 $m^2$) of each plot by cutting plants at a height of ~ 5 cm above ground level. In $S_1$, samples amounting to approximately 300 kg of fresh biomass were collected as early as April 21st, and in $S_2$, samples amounting to approximately 150 kg of fresh biomass were collected on May 3rd. The dry matter (DM) per hectare was determined in both $S_1$ and $S_2$ by drying approximately 30 kg of fresh biomass at 105 °C. From the same plants identified in the field, the marketable capitula were collected during both $S_1$ and $S_2$ to establish the percentage of residual biomass in the total biomass. The capitula thus collected were then dried at 105 °C to determine the DM.

## Laboratory Experiment

### Raw Material Preparation and Dry Matter Determination

Samples of field residues collected in $S_1$ were oven dried at 45 °C to reduce their moisture content to less than 10%. They were then milled using a laboratory hammer mill (Retsch GmbH & Co. KG, Germany) and sieved to obtain particles < 2 mm for biomass pretreatment and saccharification (Fig. 3). The DM content was determined by drying ~ 1000 mg of biomass in an oven at 105 °C until reaching a constant weight.

### Chemical Characterization of Biomass

Characterization required further reduction of the biomass particle size between 0.25 and 0.4 mm. Following the same procedure as indicated in [7] the biomass thus obtained was subjected to Soxhlet water extraction and then Soxhlet ethanol extraction. The extractive-free samples underwent the procedure for the determination of their structural carbohydrates and lignin. Extractives and polymeric carbohydrates hydrolyzed into monomeric forms were analyzed by highperformance liquid chromatography (HPLC). All assays were performed at least in duplicate.

### Liquid Solid Ratio in Water Extraction and Dilute Acid Hydrolysis Conditions

In the pretreatments, a solid phase, biomass, and a liquid phase, dilute acid solution were used. For this reason, it was necessary to find a suitable liquid/solid ratio (LSR) to avoid the liquid phase being totally absorbed by the solid phase and, on the other hand, to avoid an excessive waste of water and consequent sugar dilution. After a series of tests and following previous experiences [32], a set of tests ranging from 10 to 20 LSR was performed, and the most suitable ratio to use in the DAH treatment for OF1 biomass was found to be LSR of 15. Furthermore, a water extraction (WE) has been developed to be carried out upstream of the DAH and aimed at removing simple sugars that can be transformed into fermentation inhibitors during DAH. In this preliminary WE, the LSR adopted was 10 for 15 min at 100 °C in closed 500-mL Schott flasks capped with Teflon seals. Dried

water-extracted globe artichoke biomass was introduced to a mini-bioreactor, and a suitable volume of 2% (w/w) sulfuric acid was added to acquire an LSR = 15, with a final volume of 200 mL, to guarantee proper agitation during the following steps. DAH pretreatment lasted 60 min at 121 °C. After WE or DAH, recovered solids were weighed, and their percentage of the total dry weight was calculated. All experiments were performed at least in duplicate. For fermentation assays, the slurry was maintained untouched until use.

**Enzyme Activities and Enzymatic Digestibility**

Enzyme activity and enzymatic saccharification were performed as indicated in [32]. Cellulase from *Trichoderma reesei* (Celluclast 1.5 L) and cellobiase from *Aspergillus niger* (Novozyme 188) activities were found to be 74 FPU/mL and 970 *p*NPGU/mL, respectively. The enzymatic digestibility was carried out in 72 h at 50 °C and 150 rpm, with 60 FPU/g cellulose of cellulase and 65 *p*NPG/g cellulose of cellobiase in an orbital shaker (TEQ, JTC, Portugal), to water extracted and pretreated biomass. All assays were performed at least in duplicate. The yield of saccharification (%) of both glu- cans and xylans was obtained as indicated in the following formulas. The glucose and xylose values obtained with saccharification were multiplied by the respective anhydrous correction factors (0.9 and 0.88). The denominators of the formulas are the total contents of glucans and xylans of the corresponding biomass (untreated and pretreated):

$$\text{Saccharification yield (\%) of glucans} = \frac{\text{mass of glucose obtained} \times 0.9}{\text{mass of glucan}} \times 100 \quad (1)$$

$$\text{Saccharification yield (\%) of xylans} = \frac{\text{mass of xylose obtained} \times 0.88}{\text{mass of xylan}} \times 100 \quad (2)$$

**Saccharification and Co-fermentation**

Preparation for saccharification and fermentation was performed in a laminar flow chamber as a sequential step in the mini-bioreactor after the pretreatment step. The experimental procedure to perform enzymatic hydrolysis and fermentation is shown in Fig. 2. The assay was performed in duplicate.

***Saccharification Step*** After cooling the mini-bioreactors with DAH slurry, the pH was corrected to 4.8 by the addition of 8 N KOH. A stock solution of sodium citrate to reach a final concentration of 50 mM was added. Cellu- clast 1.5 L and Novozyme 188 were added to have final activities of 45 and 7 FPU and CBU per g of DM, respectively. A magnetic cross bar was added to each reactor, and agitation was assured with a magnetic stirrer. Saccharification was performed in a water bath at 50 °C and 200 rpm for 24 h.

***Co-fermentation Step*** Pre-inoculum was obtained by activating 2-mL frozen ethanologenic *Escherichia coli* MS04 in 40% (w/w) glycerol to a test tube with 3-mL Luria Ber- tani broth supplemented with 10-g/L xylose and kanamycin (30 mg/L) for 2 h at 300 rpm and 37 °C. The inoculum was prepared by transferring the content of the test tube to a 400-mL mini-bioreactor [35] containing 200-mL AM1 medium [36] supplemented with xylose (20 g/L), kanamycin (30 mg/L), and betaine for 18 h at 37 °C, 100 rpm and pH 7, which was maintained by the automatic addition of 2 N KOH. AM1 composition and supplements were the same as

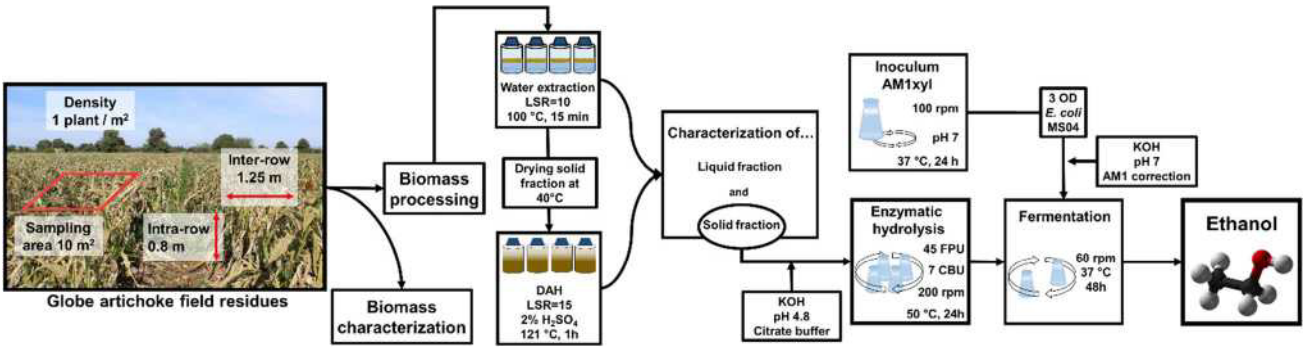



**Fig. 2** Experimental scheme for obtaining bioethanol from the field to the bioreactors. DAH dilute acid hydrolysis; EH enzymatic hydrolysis

those used by Fernandes et al.[37], but glucose was substituted by xylose. The optical density (OD) of the inoculum was read by a spectrophotometer at a 600-nm wavelength.

Prior to the addition of inoculum to each bioreactor containing the slurry from saccharification, the pH was adjusted to 7 by the addition of 8 N KOH. Stock solutions to give the same composition as the AM1 media with the exception of sugars were added to the slurry.

The appropriate inoculum volume to reach a total of 3

OD was recovered by centrifugation at 5000 x g (Hermle Labortechnik Z 323 K, Wehingen, Germany) for 10 min at 4 °C and resuspended in the slurry of each bioreactor. Finally, the mini-bioreactors were placed in a water bath at 37 °C and 60 rpm, and the pH was maintained at 7 by the controlled addition of 2 N KOH. Samples were taken every 2 h for 12 h and then every 6 h up to 48 h. Each sample was then centrifuged at 15,000 x g, frozen and analyzed by HPLC.

The fermentation efficiency (%), shown in Table 5, was obtained as a percentage ratio between the ethanol actually obtained and the ethanol theoretically obtainable from the complete fermentation of the glucose, arabinose, and xylose contained, respectively, in the glucan and hemicellulose of the biomass. Volumetric ethanol productivity ($Q_{EtOH}$) (g/L/h) was calculated as follows:

$$Q_{EtOH} = \frac{\text{ethanol produced}(g/L)}{\text{Time}\ (h)} \quad (3)$$

where produced ethanol is the obtained concentration of ethanol at time t.

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### High-performance Liquid Chromatography

A Merck Hitachi HPLC system (Tokyo, Japan) equipped

with refraction index (L7490) and UV-VIS (L7420) detectors were used to determine sugars, organic acids, and furanic compounds (furfural and 5-hydroxym- ethylfurfural) in hydrolysates. Quantitative characterization was carried out using an Aminex HPX-87H (300 X 7.8 mm) cation exchange column from BioRad (Hercules, CA, USA) at 50 °C with 5 mM $H_2SO_4$ at a flow rate of 0.4 mL/min. The same column was employed at a flow rate of 0.6 mL/min for determining hydrolysates composition from DAH, saccharification and fermentation. Sugars were also determined by means of a Rezex RPM-Monosaccharide (300 X 7.8 mm) column, with neutral HPLC grade water as the mobile phase and a flow rate of 0.6 mL/min at 75 °C for biomass characterization. External standard calibration curves were made for each analyzed compound.

### Statistical Analysis

The aboveground biomass per hectare and its distribution by plant parts and fermentable sugars obtained by enzymatic hydrolysis were subjected to analysis of variance. The means were separated by Fisher's least significance difference test, applying a threshold of 0.05. Values recorded as percentages were subjected to angular transformation prior to the analysis of variance.

## Results and Discussion

### Aboveground Residual Biomass Production and its Characterization

Figure 3 shows that the globe artichoke aboveground residual biomass was significantly more abundant in $S_1$ than in $S_2$ (16.8 t DM/ha vs. 11.9 t DM/ha). This difference is reflected in the production of capitula, which was greater in $S_1$ than in $S_2$ (3.9 t DM/ha vs. 3.1 t DM/ha). This notable difference could be explained by the low temperatures recorded in $S_2$, with at least 8 days when the temperature dropped below zero [33]. To obtain an idea of the productivity of the globe artichoke in terms of residual biomass, it can easily be compared with that of other crops such as wheat, barley, rye, oat, maize, rice, chickpea, lentil, cotton, sunflower, soybean, and tobacco. These crops typically leave less than 5 t DM/ha of postharvest residues [38].

The quantity of biomass depends on the environmental conditions and cultivation practices, as shown in Fig. 3. Composition can also change from 1 year to the next, but the major components do not vary significantly [7]. As shown in Table 1, the percentages of each of the main fractions (leaves and stems) of the residual biomass did not vary significantly from $S_1$ to $S_2$. Only the percentage of capitula changed significantly (7.6% in $S_1$ vs. 13.1 in $S_2$) for reasons related to market demand.

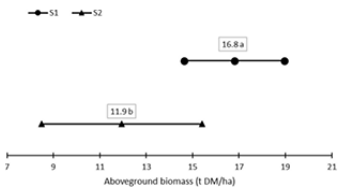



**Fig. 3** Aboveground biomass per hectare of each year with 95% confidence intervals for the mean estimate. Different letters indicate significance at the LSD test ($p \leq 0.05$)

**Fig. 3** Aboveground biomass per hectare of each year with 95% confidence intervals for the mean estimate. Different letters indicate significance at the LSD test ($p < 0.05$)

**Table 1** Percentage of each part of OF1 residual biomass on total dry weight, as affected by season. *NS* not significant; * significant at $p < 0.05$

| | | $S_1$ | $S_2$ | Significance |
|---|---|---|---|---|
| Leaves[a] | % | 34.9 | 31.4 | NS |
| Capitula[a] | % | 7.6 | 13.1 | * |
| Stalks[a] | % | 57.5 | 55.5 | NS |

[a]on total dry weight

**Table 2** OF1 biomass chemical composition expressed as % of dry matter ± standard deviation

| Component | % of total dry biomass ± standard deviation |
|---|---|
| Ashes | 9.4 ± 0.1 |
| EE | 3.0 ± 0.1 |
| AE | 29.8 ± 0.6 |
| Glucose[a] | 3.1 ± 0.2 |
| Fructose[a] | 5.3 ± 0.2 |
| Glucan | 23.8 ± 1.5 |
| Hemicellulose | 17.5 ± 0.9 |
| Xylan | 12.7 ± 0.6 |
| Arabinan | 3.3 ± 0.1 |
| Acetyl group | 1.4 ± 0.2 |
| KL | 10.5 ± 0.2 |
| Others | 6.0 ± 2.5 |

*EE* ether extract, *AE* aqueous extract, *KL* Klason lignin [a] present in the water extract as soluble sugar

The percentage of residual dry biomass in $S_1$ was approximately 80% of the total (i.e., that including harvested capitula), while that obtained in $S_2$ was approximately 77%; these are high percentages because OF1 is a hybrid variety and therefore quite vigorous. All this seems to confirm that seasonal trends can decisively influence the quantity of biomass but not so much the proportions of its parts. For this reason, the crop residues of only 1 year, precisely $S_1$, were characterized and subjected to the experiments. The contents of glucans (23.84%) and hemicelluloses (17.48%) (Table 2) in
OF1 residues found in this study are consistent with those found by Pesce et al. [32] in globe artichoke. Cotana et al. [30] and Fernandes et al. [31] conducted studies in cultivated cardoon, which belongs to the same species as globe
artichoke, but found quite different values of cellulose and
hemicellulose (35-42% and 13-16%, respectively). However, these latter values were obtained from samples collected after the summer, i.e., long after flowering and when the biomass was already dry. In another comparison, the glucan content of stover from white corn is 30%, while the hemicelluloses amount to 19.6% [39].

Not surprisingly, globe artichoke post-harvest biomass contains fructose (5.29%) (Table 2). The plant is in fact rich in inulin, a polysaccharide that accumulates in its reserve organs, such as the receptacle [40, 41]. Under certain pH and temperature conditions,

inulin is hydrolyzed to fructose [42]. In addition to fructose, the biomass of OF1 also contains glucose (3.14%) (Table 2). This fact has consequences because the simple sugars present in the biomass can be transformed into inhibitors during pretreatments. Since the aim of this study was to produce ethanol directly from the slurry obtained from the pretreatment, passing through enzymatic hydrolysis, it was necessary that the starting biomass be free of soluble sugars.

### Liquors Deriving from WE and DAH

Liquors derived from WE and subsequent DAH were analyzed to determine monosaccharides and decomposition side-products (acetic acid, furfural, and hydroxymethylfur- fural) (Table 3). The liquid fraction obtained with WE was rich in glucose (4.5% of DM) and fructose (5.3% of DM). Since the temperature used to perform the water extraction was not above 100 °C, this did not degrade the simple sugars contained in the biomass and consequently did not cause the formation of furans. WE was expressly aimed at obtaining a biomass free of simple sugars that could be transformed into inhibitors [43, 44] in dilute acid hydrolysis. In the liquid fraction derived from DAH, there was no fructose, and the content of glucose (0.8% DM) was very low because the biomass had previously been subjected to WE, with which simple sugars were removed. DAH led to the depolymerization of the hemicelluloses, with the formation of pentoses, namely, xylose (14.2% of DM) and arabinose (2.7% of DM) (Table 3). The DAH was severe enough to cause breakdown of the hemicellulosic fraction and simple sugar degradation to generate acetic acid and a small quantity of furans (Table 3).

**Table 3** Monosaccharides and decomposition side-products of liquid fractions obtained with water extraction (WE) and with subsequent dilute acid hydrolysis (DAH) on OF1 biomass. The results are expressed as % of dry matter

| Pret | Components (% of DM) | | | | | | |
|---|---|---|---|---|---|---|---|
| | GLU | XYL | FRU | ARA | AcA | HMF | FUR |
| WE | 4.5 ± 0.0 | ND | 5.3 ± 0.0 | ND | ND | ND | ND |
| DAH | 0.8 ± 0.0 | 14.2 ± 0.1 | ND | 2.7 ± 0.0 | 4.2 ± 0.1 | 0.3 ± 0.0 | 0.2 ± 0.0 |

*WE* water extraction, *DAH* dilute acid hydrolysis, *DM* dry matter, *GLU* glucose, *XYL* xylose, *FRU* fructose, *ARA* arabinose, *AcA* acetic acid, *HMF* hydroxymethylfurfural, *FUR* furfural, *ND* not detected

The xylose yield in the liquid fraction was higher than that obtained by Ballesteros et al. [8], where a maximum of 13.5 g/100 g of cardoon was reached with a 7.5% (w/v) solid concentration, 180 °C and 0.1% acid (w/v). It was also higher than that obtained by Tavares et al. [10], which amounted to 8.11 g/100 g of cardoon with LSR 10, 6.7% acid (w/w) at 121 °C for 55 min. A similar xylose quantity was obtained with the cv. Spinoso sardo of globe artichoke [32]. Shatalov and Pereira [9], on the other hand, obtained a higher xylose concentration, i.e., a maximum of 18.08 g/100 g of cardoon with 1.28% sulfuric acid at 138.5 °C for 52 min, with a LSR of 15. The sugar production achieved in the present work was higher than that obtained by Cavalaglio et al. [45] with steam explosion applied to cardoon stalks, which led to a maximum of 10.5 g of xylose and 1.5 g of glucose per 100 g of raw biomass.

Due to the prior WE, the inhibitors (furfural and hydrox- ymethylfurfural) released in the liquid fraction during the DAH setup in this work reached only 0.5% of the dry matter. This value is significantly lower than those obtained by both Ballesteros et al. [8] and Tavares et al.[10]. The former, in fact, obtained a total of 3% of inhibitors distributed as follows: less than 1% furfural, 0.2% hydroxymethylfurfural, and 1.8% formic acid. The latter even obtained 5.84% of inhibitors distributed as follows: 2.19% furfural, 0.04% hydroxymethylfurfural, 3.32% formic acid, and 0.26% lev- ulinic acid. These differences can also be explained, on the one hand, by the use of higher temperatures in Ballesteros et al. [8] (180 vs. 121 °C) and, on the other hand, by the use of a higher acid concentration in Tavares et al. [10] (6.7 vs. 2% (w/w)), which produced higher quantities of formic and levulinic acids formed from the further degradation of furanic compounds [43]. Shatalov and Pereira [9], also produced higher inhibitor concentrations (1.04% furfural, 0.33% hydroxymethylfurfural) in comparison with the present work for the same reasons explained as for the Ballesteros [8] case, i.e., because of the higher temperature used during the pretreatment and the presence of soluble sugar in cardoon.

### Pretreated Solid Fractions

The chemical composition of OF1 biomass from WE and dilute acid hydrolysis is shown in Table 4. The solids from water extraction and those from acid hydrolysis show marked differences, as might be expected, except for the recovered solids (72.2% vs. 69.8%) (Table 4). This could suggest that DAH does not involve a large loss of biomass. The explanation is that DAH is performed after the WE, so the biomass subjected to DAH has already lost some of its solids in the
previous step (WE). This means that the real percentage of
recovered solids after DAH is 0.722 x 0.698 x 100 = 50.4%. Solids from DAH have, on the one hand, higher percentages of glucans than solids coming from WE (52.5% vs. 34.3% of DM) but, on the other hand, have a lower percentage of hemicellulose (14.1% vs. 25.9% of DM) (Table 4). The latter, in fact, was partially depolymerized during DAH and released in the form of xylose and

acetic acid in the liquid fractions. Table 5 shows fermentable sugars obtained by the enzymatic digestibility test performed on solid fractions from each pretreatment. Glucose and xylose yields, expressed as a percentage of cellulose and xylan, respectively, were higher after DAH than after WE. It is interesting to note that the biomass of OF1 responded to saccharification much better than the biomass of cv. Spinoso sardo studied in Pesce et al. [32]. In fact, the biomass of OF1 subjected to DAH after WE had a yield close to 100% of obtainable glucose (Table 5) versus ~ 55% of "Spinoso sardo." By applying DAH to the biomass of cultivated car- doon, which, as mentioned, belongs to the same species as the globe artichoke, Ballesteros et al. [46] obtained a saccharification yield of 80.2%, while Shatalov and Pereira [9] and Fernandes et al. [7] achieved saccharification yields of 76% and 68%, respectively. Xylan yields were significantly different (20.9% for WE vs. 44.0% for DAH). Glucan and Xylan yields, expressed as kg per t DM, are shown in Table 5 as well. They amounted to 167 and 581 kg of glucose per t DM for WE and DAH, respectively, and 45 and 69 kg of xylose per t DM for WE and DAH, respectively (Table 5). The yields of glucose and xylose, expressed as kilograms per hectare, are also shown in Table 5. Glucose yielded from WE biomass is less than half of that yielded from DAH (2029 vs. 4917 kg/ha), while the yields of xylose of the two pretreatments are not so different (541 vs. 585 kg/ha) (Table 5). To make a comparison with one of the most suitable species for the production of bioethanol, the biomass of sweet sorghum (*Sorghum bicolor* L. Moench) has a sugar yield that can vary from 4 to 17 t/ha.[47].

Pretreatment Component (% of total DW ± standard deviation)

**Table 4** Chemical composition of solid fractions of OF1 biomass obtained after the pretreatments. Values are expressed as % of dry matter

| | Ash | Glucan | Hemicellulose | KL | Others | RS |
|---|---|---|---|---|---|---|
| WE | 6.7 ± 0.9 | 34.3 ± 0.2 | 25.9 ± 0.1 | 19.8 ± 1.2 | 13.3 ± 1.2 | 72.2 ± 0.6 |
| DAH | 1.2 ± 0.4 | 52.5 ± 0.7 | 14.1 ± 0.2 | 27.0 ± 1.9 | 5.3 ± 1.6 | 69.8 ± 1.0 |

*WE* water extraction, *DAH* dilute acid hydrolysis, *DW* dry weight, *KL* Klason lignin, *RS* recovered solids (after pretreatment)

**Table 5** Fermentable sugars obtained by the enzymatic hydrolysis of solid fractions from each pretreatment. Percentage yields show how much simple sugars are obtained compared to the maximum obtain-
able from the complete depolymerization of polysaccharides. Different letters within each variable indicate significance at LSD test $p < 0.05$)

| Pret | Glucan yield % | Xylan yield % | kg GLU/t DM[a] | kg XYL/t DM[a] | kg GLU/ha | kg XYL/ha |
|---|---|---|---|---|---|---|
| WE | 43.9 B | 20.9 B | 167 B | 45 B | 2029 B | 541 B |
| DAH | 99.6 A | 44.0 A | 581 A | 69 A | 4917 A | 585 A |

*WE* water extraction, *DAH* dilute acid hydrolysis, *GLU* glucose, *XYL* xylose [a] of recovered solids after pretreatment

## Saccharification Study

The evolution of glucose and xylose generated during 24 h of saccharification before the fermentation step, together with saccharification yields in glucan and xylan, are represented in Fig. 4. After 24 h of enzymatic hydrolysis, a glucan yield of 69% was attained (Fig. 4b), this value is notably lower than the 80% obtained by Shatalov and Pereira [9] with analogous treatment (DAH at 130 °C) applied to cardoon. This gap is explained primarily because Shatalov and Pereira [9] applied significantly higher concentrations of enzymes, namely, 60 FPU and 64 *p*NPG/g cellulose of cellulase and cellobiase, respectively. Furthermore, the presence of the liquid fraction could have a negative effect on saccharification. In fact, the monosaccharides (xylose, arabinose, and glucose), furan compounds (HMF and furfural) (Table 3) and phenolic compounds generated by the solubilization of the lignin present in the slurry can inhibit cellulolytic enzymes [48]. In this regard, Cavalaglio et al. [45] also observed a significant reduction in enzymatic saccharification in the presence of the liquid fraction (from 76 to 58%). In light of these considerations, it can be affirmed that in this experiment, even if there was an inhibition of the enzymatic activity, this was nevertheless not such as to compromise the sugar yields. The present work obtained a higher saccharification yield, and the obtained solubilized xylose was also higher (14.5%) (Table 3) than the results of Cavalaglio et al. [45] (10%).

## Fermentation Study

The enzymatic slurry was fermented by ethanologenic *E. coli* MS04 for 48 h. The dynamics of fermentation over time are described in Fig. 5. The ethanol content of the mini-bioreactors increased as glucose was rapidly consumed in the first 8 h, indicating that the strain used was not affected by the presence of acetic acid and inhibitors. Glucose dynamics is the inverted photogram of the ethanol content. The glucose content, in fact, clearly drops for 8 h, and its curve flattens out. The OD inoculated was very effective for ethanol conversion, allowing it to reach 10.93 g/L at 8 h. with a volumetric ethanol productivity of 1.37 g/L/h. Only after having completely consumed the glucose did *E. coli* MS04 begin to use xylose, which indicates its preference for hex- oses over pentoses.

This behavior was already observed for *E. coli*, both wild-type and metabolically engineered, and is known as carbon catabolite repression, in which the microorganism, in the presence of a mixture of sugars in the biomass hydrolysates, consumes glucose first and then other sugars such as arabinose and xylose. This often results in a delayed and incomplete consumption of the secondary sugars [49]. The initial concentration of xylose in the slurry was approximately 8.5 g/L, and the concentration at 24 h was approximately 3.7 g/L. Finally, the concentration at 48 h was

**Fig. 4** Kinetics of enzymatic saccharification with 45 FPU and 7 CBU per g DM of OF1 pretreated with DAH (2% $H_2SO_4$, 121 °C, 1 h). **a** Shows the glucose and xylose concentration values (± standard deviation). **b** Shows saccharification yields in glucan and xylan expressed as percentages (± standard deviation)

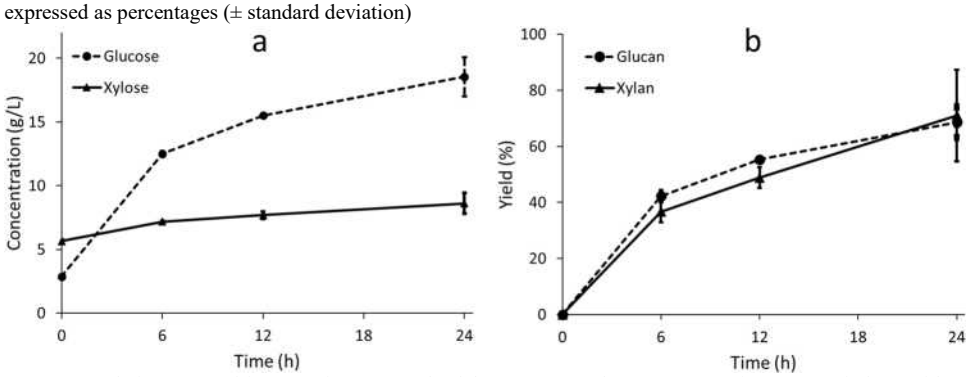
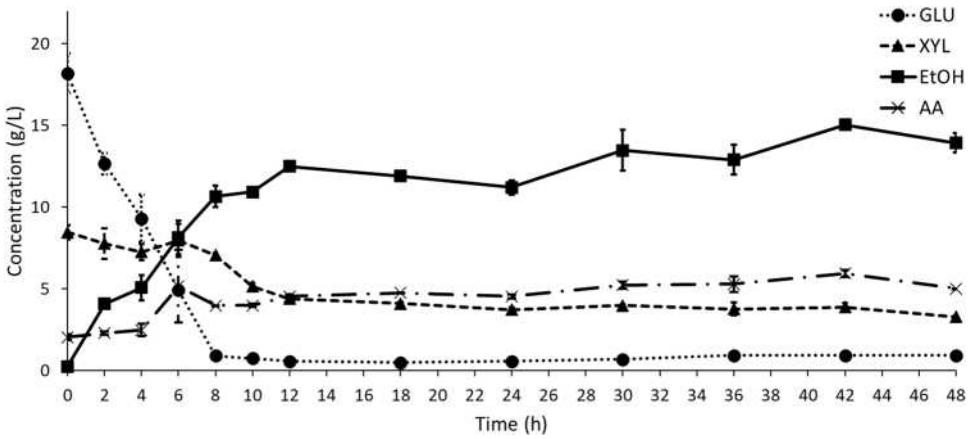



**Fig. 5** Cumulative curve of fermentation over 48 h of the OF1 DAH slurry. Fermentation was carried out with *E. coli* MS04. Vertical bars indicate standard deviation. GLU glucose, XYL xylose, EtOH ethanol, AA acetic acid




approximately 3.3 g/L, which means that 56% of xylose was consumed 24 h after the start of fermentation, while it was 61% after 48 h (Fig. 5). Similar behavior was observed in a previous study performed with MS04 with olive tree pruning pretreated with phosphoric acid [50]. This low consumption of xylose could be improved by performing fermentation for more time. Another possibility would be by applying a detoxification step to remove any phenolic compound that could inhibit the fermentation process, as was shown in the fermentation of detoxified hydrolysate from cardoon [10] which allowed a rapid and complete fermentation of xylose and glucose. On a larger scale, studies have been conducted to overtake carbon catabolic repression. Fernandez-Sandoval et al. [49] performed two-stage continuous cultures under micro-aerated conditions that allowed the total co-consumption of a mixture of glucose and xylose with an increase of 23% volumetric ethanol productivity in comparison to the batch stage and 78% with single-stage continuous cultures. Additionally, arabinose (not shown in Fig. 5) was present at the beginning of fermentation, but in a very low quantity (1.43 ± 0.26 g/L) that was consumed after 36 h. Acetic acid did not affect the performance of fermentation and was also produced during the fermentation process, passing from 2 to 5 g/L. Table 6 shows some parameters related to bioethanol production at 24 and 48 h from the start of fermentation. The fermentation efficiency was 76% at 24 h from the start of fermentation, while 48 h after the start of fermentation was 86% (Table 6); it is expressed as a percentage of ethanol obtainable from the theoretical complete fermentation of glucose and xylose achievable from the complete depolymerization of cellulose and hemicellulose.

The concentrations of ethanol in the slurry at 24 and 48 h from the start of fermentation were similar (12.5 vs. 14.1 g/L). The volumetric ethanol productivity was 0.52 g/L/h in the first 24 h and 0.29 g/L/h in 48 h (Table 6). The ethanol yield per biomass unit

was 283 kg/t DM after 24 h of fermentation and reached 321 kg/t DM after 48 h of fermentation (Table 6). This last value is compatible with

**Table 6** Ethanol production values (±standard deviation) obtained after 24 h and 48 h of fermentation applied to biomass previously subjected to DAH

| Parameter | UM | 24 h | 48 h |
|---|---|---|---|
| Fermentation efficiency | % | 76.0 ± 1.9 | 86.0 ± 0.9 |
| Ethanol concentration | g/L | 12.5 ± 0.3 | 14.1 ± 0.2 |
| Ethanol yield per biomass unit | kg/t DM[a] | 283 ± 7.1 | 321 ± 3.5 |
| Ethanol yield per area unit | kg/ha | 2399 ± 60 | 2715 ± 30 |

*UM* unit of measure
[a] refers to biomass as it is, i.e., before WE and DAH

the theoretical bioethanol calculated starting from the fermentable sugars obtained from the saccharification of the solid fraction of the biomass coming from DAH (Table 5). In fact, the sum of 581 kg/t DM of glucose and 69 kg/t DM of xylose, multiplied by the coefficient 0.51 (based on the theoretical yield of 0.51 g ethanol/g of sugar), is 331.5 kg EtOH/t DM. The ethanol yield per hectare was 2399 kg after 24 h and 2715 kg after 48 h. This last value is similar to that calculated with the glucose and xylose values found in Table 5. In fact, the sum of 4917 kg/ha of glucose and 585 kg/ha of xylose, multiplied by 0.51, gives 2806 kg/ha of ethanol. For comparison, sweet sorghum, which is one of the most productive species for ethanol production, can yield up to 6000 L of ethanol per hectare [47].

The fermentation strategy allowed for obtaining a higher fermentation efficiency (76 and 86% for 24 and 48 h, respectively) than those obtained in previous work performed with cardoon pretreated with DAH, which was 65% both in Fernandes et al. [7] and Ballesteros et al. [46]. The observed high efficiency could be explained by the rapid uptake of glucose to be converted to ethanol by the ethanologenic *E. coli* strain MS04; indicating that a small amount of sugar was used for microorganisms to grow since MS04 was applied to the enzymatic slurry when the strain was growing in the exponential phase at a high rate. The values of ethanol obtained here were slightly higher than 11.5 and 12.2 g/L for the SHF (separate hydrolysis and fermentation) and SSF (simultaneous saccharification and fermentation) experiments of Fernandes et al. [7]. Ethanol concentration was lower than the 23 g/L attained by Ballesteros et al. [46] with SSF, but this can be explained by the ~5% solid loading used in the present study, significantly lower than the 10% used by the above-mentioned authors. Vargas-Tah et al. [39] used *E. coli* MS04 with stover from white corn and produced 24 g/L ethanol with 74-76% fermentation efficiency after 24 h of fermentation. Although they achieved a higher ethanol concentration, due to the use of a higher solid loading (15%), the overall process was equivalent to the results here.

## Conclusions

The post-harvest biomass of a hybrid variety of globe artichoke was evaluated for bioethanol production. In the 2-year field trial, the biomass values recorded were significantly different due to the different thermo-pluviometric conditions. The biomass characterization revealed the presence of simple sugars, which can be transformed into fermentation inhibitors during dilute acid hydrolysis (DAH) pretreatments. Therefore, water extraction (WE) was performed before DAH to remove all soluble sugars. The biomass thus obtained, subjected to DAH, produced few inhibitors, and the subsequent enzymatic saccharification resulted in a high yield of glucans. Ethanolic fermentation of the saccharified slurry was performed by the *E. coli* strain MS04. The ethanol yield after 24 h of fermentation was 283 kg/t DM with ability to generate 2399 kg of ethanol per hectare. These results are promising, but further studies are needed to increase fermentable sugars release from polymers, decrease inhibitor formation and maximize global ethanol yield.

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**Data Availability** All data generated or analyzed during this study are included in this published article.

## Declarations

**Competing Interests** The authors declare no competing interests.

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