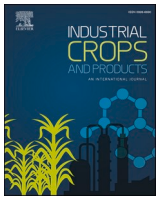
Industrial Crops & Products 214 (2024) 118570

Contents lists available at ScienceDirect

Industrial Crops & Products

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



Exploitation of cardoon roots inulin for polyhydroxyalkanoate production

Iolanda Corrado [a,1], Elisabetta Borselleca [a,1], Giovanni Dal Poggetto [b], Ivana Staiano [a], Maria Laura Alfieri [a], Cinzia Pezzella [a,c,*]

[a] Department of Chemical Sciences, University "Federico II", Naples, Italy
[b] Institute for Polymers, Composites and Biomaterials, IPCB CNR, Pozzuoli, Naples, Italy
[c] Consorzio Interuniversitario Reattività Chimica e Catalisi (CIRCC), Bari 70121, Italy

ARTICLE INFO

Keywords:
Biorefinery crop
Microbial biopolymer
Simultaneous saccharification and fermentation
Inulinase, *Cupriavidus necator* DSM 545
Burkoldheria cepacia DSM 50181

ABSTRACT

Cynara cardunculus, cardoon, is a biorefinery crop with an overwhelming role in the bioplastic scenario. This work explored the use of inulin extracted from cardoon roots as a feedstock for polyhydroxyalkanoates (PHA) production. Cardoon roots from both spring and winter seasons were subjected to two protocols consisting of an autoclave extraction followed by *i)* an ethanol precipitation and further lyophilization or *ii)* lyophilization directly. The resulting extracts were characterized for recovery yield (from 11.6 to 16 g of inulin per 100 g of roots), purity grade (from 64% to 97%) and molecular weight distribution, the latter being affected by both seasonal variability and the extraction method. The performances of two PHA producers, *Cupriavidus necator* and *Burkholderia cepacia*, were compared in Simultaneous Saccharification and Fermentation of spring inulin extracts obtained with the two protocols, exploring the effect of controlled addition of fungal inulinase *PlaI*. Up to 2 g/L of polyhydoxybutyrate (PHB) polymer was produced in the best feeding condition, with both strains found able to metabolize the main phenolic acids coextracted with inulin. Diversity in polymer yields were observed, with evidence of the synthesis of PHB polymers characterized by different molecular weight distributions depending on the type of feeding and microorganism employed. The proposed processes are placed in the frame of the circular economy approaches applied to the valorization of cardoon biomass in the bioplastic field.

1. Introduction

The switch towards a fossil-based economy towards a more bio-based one takes shape into the development of biorefineries, industrial facilities intended for the sustainable processing of biomass into a spectrum of bio-based products (food, feed, chemicals, materials) and bioenergy (biofuels, power, and/or heat) (Calvo-Flores and Martin-Martinez, 2022).

A biorefinery must first rely on the high availability of biomass in a concentrated area, to reduce the transportation costs and to add value to the rural economies. The valorization of local agro-forestry residues together with crops that do not compete with the food chain is a viable way to address biorefinery sustainability (Vergara et al., 2018).

Cardoon (*Cynara cardunculus* L., Asteraceae family) is a perennial herbaceous species of the Mediterranean region, widely recognized as one the most promising biorefinery crops of this area. This annual plant is a non-food oilseed crop, fully adapted to soils with water stress and large variations in temperature, and is characterized by high biomass productivity in marginal lands (about 9–26 t/ha of dry biomass estimated in Spain, Italy, France and Greece). Italy, in particular, hosts industrial initiatives, led by Novamont and Versalis respectively, aimed at the fully exploitation of this crop (Turco et al., 2022).

Cardoon biomass has been proved as a raw material in pulp and paper production, power generation, as well as in a number of valorization approaches leading to added-value products, such as biofuels, bio-lubricants, bioplastics and active ingredients for cosmetic and pharmaceutical uses (Ciancolini et al., 2013; Cotana et al., 2015; Gominho et al., 2018; Mauromicale et al., 2019). The fractionation of this biomass into individual components (seeds, leaves, stems, roots) and their channeling into a dedicated valorizing path, is an effective approach to preserve the value of each fraction. A step forward has been represented by holistic approaches in which the bioproducts obtained from each dedicated path have been recombined into fully-biobased products, such as upgraded bioplastics. Emblematic examples are

* Corresponding author at: Department of Chemical Sciences, University "Federico II", Naples, Italy.
E-mail address: cpezzella@unina.it (C. Pezzella).
[1] Equally contributing authors.

https://doi.org/10.1016/j.indcrop.2024.118570
Received 17 November 2023; Received in revised form 18 March 2024; Accepted 13 April 2024
Available online 17 April 2024

represented by the development of active films based on proteins extracted from the oilseed cake and the bioactive molecules from the leaves (Mirpoor et al., 2022) and more recently, by the formulation of polyhydroxyalkanoates-based materials with epoxidized cardoon oil as additive (Turco et al., 2022).

Cardoon biomass has been also considered a valuable source of inulin, a fructan constituted by fructose units linked by β-(2−1) linkages, widely used in a number of food and non-food applications, depending on its degree of polymerization (Branca et al., 2022; Raccuia et al., 2004). Several works have found high levels of inulin accumulated in cardoon roots as an adaptive strategy to overcome the unfavorable Mediterranean conditions. Furthermore, a recent study based on multi-criteria decision analysis (MCDA) identified *C. cardunculus* as the most promising biomass for the extraction of inulin in terms of strategic factors affecting profitability and environmental impact (Borroni et al., 2021).

Inulin-rich feedstocks have been explored as renewable source to produce different microbial products, such as biofuels and biopolymers, employing different bioprocess strategies, including Separate Hydrolysis and Fermentation (SHF), Simultaneous Saccharification and Fermentation (SSF) and Consolidated Bioprocesses (CBP) (Singh et al., 2022). Among biopolymers, polyhydroxyalkanoates (PHA) are microbial polyesters emerged as valid alternative to fossil-based bioplastics due to their proved biodegradability in soil and marine environments and their tunable material properties, which depend on the type of biocatalyst involved and the nature of the C-source used for their synthesis (Turco et al., 2021). As a fact, PHA is an heterogenous family of polymers comprising short chain length PHA (scl-PHA) and medium chain length PHA (mcl-PHA) based on the total number of the carbon atoms of the monomers. This diversity results in polymers with different thermal and mechanical properties, which have been exploited in different applications, *i.e.* food packaging, biomedical implants for tissue engineering, agricultural sectors for the delivery of herbicides (Corrado et al., 2023, 2021c; Hossain et al., 2022; Kalia et al., 2021).

Recent findings suggest the use of inulin as an effective C-source for PHA production, allowing to address the issue of the high costs of the carbon sources required for the fermentation process (Corrado et al., 2023). As a fact, inulin from chicory roots or from Jerusalem artichoke tubers proved to be effective in allowing polyhydroxybutyrate (PHB) production in both SHF and SSF processes catalyzed by *C. necator* species. In both these processes, inulin hydrolysis into fermentable sugars, catalysed by microbial inulinases, was a mandatory step (Corrado et al., 2021a; Turco et al., 2022). In another example, a co-culture based system has been implemented to circumvent the need for the addition of inulinase, introducing an inulinase producing strain into the microbial consortium (Corrado et al., 2021b).

The aim of this work is to assess the feasibility of microbial PHA production from inulin extracts from cardoon roots, verifying the effect of the extraction methods on the performance of two PHA producers: *Cupriavidus necator* DSM 545 and *Burkoldheria cepacia* DSM 50181. While the former was chosen as a reference strain thanks to its verified ability to produce PHB from inulin hydrolysates (Haas et al., 2015), *B. cepacia* was selected due to its ability to metabolize different sugar sources and its tolerance towards different inhibitory compounds (including those deriving from the treatment of lignocellulose materials) (Pan et al., 2012b). To the best of our knowledge, this is the first report exploring inulin extracts from cardoon roots as raw material for PHA production.

2. Materials and methods

2.1. Plant material

Cynara cardunculus roots were provided by Novamont S.p.A. from Terni (42.561335 N latitude, 12.62860 E longitude) (Umbria Region, Central Italy). The roots were harvested in December 2020 and May 2021. After cleaning, they were manually chopped and dried at 60°C until they reached constant weight (Pari et al., 2021). They were stored for three months at room temperature until the extraction step.

2.2. Microbial strains and culture conditions

Cupriavidus necator DSM 545 and *Burkholderia cepacia* DSM 50181 were purchased from the German Collection of Microorganisms and Cell cultures (DSMZ, Germany). Both strains were propagated aerobically at 28°C on Nutrient Broth (NB) medium.

2.3. Inulin extraction

C. cardunculus dried roots were reduced to sections of approximately 1–2 cm in size, milled in a steel blender and passed through a 0.5 mm sieve, collecting the powder < 0.5 mm. Inulin was extracted with distilled water (20% w/v) at 120°C 20 min 1 bar. After filtration, the aqueous extract was lyophilized or subjected to purification by addition of cold ethanol to favour polysaccharides precipitation. The precipitate was recovered by centrifugation at 6370 rcf and lyophilized. DL refers to the lyophilized aqueous extract, while EL to the extract purified by means of ethanol precipitation. Extracts (EL/DL) were characterized for inulin content by Fructan HK assay kit (K-FRUCHK by Megazyme).

2.4. Enzymatic inulin hydrolysis

Enzymatic hydrolysis of inulin was performed by using exo/endo inulinases mixture, *PlaI*, produced by *Penicillium lanosocoeruleum* according to Corrado, 2021 (Corrado et al., 2021a). The reactions were performed in 10 mL glass vials with 5 mL of 0.1 M Na-Acetate buffer at pH 5.1 supplemented with 60 g/L of extract and 50 $U/g_{extract}$. The vials were incubated at 45.4°C for 24 h. Samples were withdrawn at different times and used for determination of residual inulin (%) and fructose release (%) as function of time. Residual inulin (%) was determined by Fructan HK assay kit (K-FRUCHK by Megazyme), which measures both inulin and fructo-oligosaccharides content. Fructose release (%) was calculated as the ratio between the grams of fructose released and the maximum amount of fructose obtained by the total hydrolysis of the extract. The latter was separately carried out by incubating the *Aspergillus niger* endo-exoinulinase enzyme mixture (SIGMA CAS: 9025–67–6) for 4 h at 50°C (5 $U/g_{substrate}$). Afterward, the mixture was kept in 100°C boiling water for 1 h to assure the complete hydrolysis and released fructose was assayed (Corrado et al., 2021a).

2.5. PHA production and extraction

C. necator DSM 545 and *B. cepacia* DSM 50181 were precultured in 100 mL flasks containing 20 mL of Nutrient Broth, NB for 24 h. PHA production was carried out in the same volume of adequately defined minimal media (MM). The composition of the minimal media for *C. necator* strain (pH 6) was: 2.32 g/L KH_2PO_4, 2.18 g/L Na_2HPO_4, 0.25 g/L $MgSO_4$, 7.5 mg/L $CaCl_2$, 5 mg/L $MnCl_2$, 1.09 g/L NH_4Cl, 500 μL/L of trace elements solution (10 mg/L $ZnSO_4 \cdot 7\ H_2O$, 3 mg/L $MnCl_2 \cdot 4\ H_2O$, 30 mg/L H_3BO_3, 20 mg/L $CoCl_2 \cdot 6\ H_2O$, 1 mg/L $CuCl_2 \cdot 2\ H_2O$, 2 mg/L $NiCl \cdot 6\ H_2O$, 3 mg/L $Na_2MoO_4 \cdot 2\ H_2O$). The composition of the minimal media for *B. cepacia* strain (pH 6.8) was: 4.6 g/L $NaH_2PO_4 \cdot H_2O$, 4.6 g/L Na_2HPO_4, 0.45 g/L K_2SO_4, 0.39 g/L $MgSO_4$, 62 mg/L $CaCl_2$, 5 mg/L NH_4Cl, 200 μL/L of trace elements solution (15 g/L $FeSO_4 \cdot 7\ H_2O$, 2.4 g/L $MnSO_4 \cdot H_2O$, 2.4 g/L $ZnSO_4 \cdot 7\ H_2O$, 0.48 g/L $CuSO_4 \cdot 5\ H_2O$, dissolved in 0.1 M HCl). All media components were purchased from Sigma-Aldrich Corp. (St. Louis, MO). Both defined media were supplemented with 20 g/L cardoon roots extracts, i.e. Spring cardoon root treated with Direct Lyophilization method (SDL) or Ethanol precipitated and further Lyophilized (SEL), as carbon sources. The latter were previously prepared by dissolving the lyophilized powder in deionized water at a concentration of 25 g/L and sterilized by

filtration in a Stericup filter unit. Inocula were performed at an OD_{600} value of 0.1. Both precultures and cultures were carried out at 28°C and 200 rpm. *PlaI* exo/endo inulinases mixture were provided at the same time as the inoculum (t0) and after 30 h from inoculum (t30) at a concentration of 40 $U/g_{extract}$. Cells were harvested by centrifugation (6000 rpm, 20 min, 4°C), lyophilized, and weighted to determine the cell dry weight (g/L) at 24, 48 and 72 h from inoculation. Culture supernatants were collected and subjected to 3,5-dinitrosalicylic acid (DNS) assay for the determination of reducing sugars concentration (Negrulescu et al., 2012). PHA polymers were extracted from the lyophilized cells by chloroform extraction and quantified by weighting (Mirpoor et al., 2023), then stored at 4°C until further use. Residual biomass (g/L) was determined by subtracting polymer concentration (g/L) to cell dry weight (g/L). ^{1}H NMR spectroscopy on the produced polymers were performed on a Bruker DRX-400 spectrometer in $CDCl_3$ (internal standard: $CDCl_3$ at 7.26 ppm) (Corrado et al., 2021b).

2.6. Analytical methods

2.6.1. Structural carbohydrates and lignin determination

Structural carbohydrates and lignin content in cardoon roots, before and after inulin extraction, were determined according to standard biomass analytical methods provided by National Renewables Energy Laboratory (NREL) (Sluiter et al., 2008). This procedure used a two-step acid hydrolysis to fractionate the biomass into forms that are more easily quantified. Lignin fractionated into acid insoluble lignin (AIL) and acid soluble lignin (ASL), which was quantified by UV-Vis spectroscopy at 240 nm. Structural carbohydrates are hydrolyzed into the monomeric forms, which are soluble in the hydrolysis liquid and were analysed by High Performance Anion Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAD). Residual content (%) of biomass components after inulin extraction was calculated normalizing the data by the biomass weight loss resulting after the different treatments.

2.6.2. Determination of monosaccharides concentration

Monomeric sugars concentration was determined by High Performance Anion Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAD) by a Dionex ICS-5000 Ionic Chromatograph equipped with ICS-5000 SP pump, ICS-5000 ED detector and a carbohydrates separation column (CarboPac SA10, 4 mm × 250 mm, Dionex) with a preguard column (CarboPac SA10, 4 mm × 50 mm, Dionex). The operating conditions were a flow rate of 1.5 mL/min of 1 mM KOH, a 15 min run at column temperature of 45°C, an injection volume of 10 μL. Sugars identification and quantification were respectively achieved using retention times and the related sugar calibration curve ($R^2 > 0.98$). Prior to injection, samples were filtered through a single-use 0.22 μm PES syringe filter. Each sample was adequately diluted and run in triplicate.

2.6.3. Determination of reducing sugars concentration

Culture supernatants were collected at different times from inoculum and analyzed by 3,5-dinitrosalicylic acid (DNS) assay for the determination of reducing sugars concentration. Briefly, after harvesting the cells by centrifugation, 200 μL of supernatants were transferred into 1.5 mL Eppendorf tubes and 500 μL of DNS reagent (10 g/L DNS, 2 g/L phenol, 10 g/L NaOH, 0.5 g/L Na_2SO_3) were added. The samples were boiled for 15 min. Once cooled, 500 μL of potassium sodium tartrate buffer (400 g/L) were added to each tube. The absorbance of the samples was measured at 540 nm against the blank (200 μL of deionized water and 500 μL of DNS reagent). The concentration of reducing sugars in the supernatants was determined using glucose as a calibration standard (0.05–1 mg/mL). All measurements were performed in triplicate.

2.6.4. HPLC and LC-MS analysis

HPLC analyses were performed on an instrument (Agilent 1100, Cernusco sul Naviglio, Milan, Italy) equipped with a binary pump and a SPD-10AV VP UV–vis detector set at 254 nm. The chromatographic separation was achieved on a Sphereclone octadecylsilane-coated column, 250 mm × 4.6 mm, 5 μm particle size (Phenomenex, Castel Maggiore, Bologna, Italy) at 1 mL/min using binary gradient elution conditions as follows: 0.1% formic acid (solvent A)/methanol (solvent B) from 5% to 30% B, 0–20 min, 30% B, 20–40 min. LC-MS analyses were run on a LC-MS ESI-TOF 1260/6230DA Agilent instrument operating in positive ionization mode and equipped with a UV–vis detector set at 254 nm. The following conditions were adopted: nebulizer pressure 35 psig; drying gas (nitrogen) 5 L/min, 325°C; capillary voltage 3500 V; fragmentor voltage 175 V. An Eclipse Plus C18 column, 150 × 4.6 mm, 5 μm (Agilent, Cernusco sul Naviglio, Milan, Italy) was used with the same eluant as above at a flow rate of 0.4 mL/min.

2.6.5. Gel permeation chromatography

Gel permeation chromatography was performed using a GPC Max Viscotek (Malvern, UK) Phenomenex (Torrance, CA, USA) system equipped with a TDA 305 triple detector array (refractive index, low angle light scattering, right angle light scattering, and viscometer). For inulin samples, columns set was composed by a pre-column and two columns PolySep-GFC-P 5000 and 3000. Samples were dissolved in MilliQ water containing 0.02% NaN_3 and 0.1 M $NaNO_3$ (concentration ≅ 4 mg/mL) and eluted in the same solvent. After complete dissolution, the sample was filtered through 0.22 μm NY filter. The injection volume was 100 μL and the flow rate was 0.8 mL/min. The chosen method of analysis was triple point, calibrated with a Pullulan standard, provided by Viscotek, with a narrow molecular weight distribution. The measurements, performed at 35°C according to the temperatures of the columns and detectors, ran for 50 min in duplicate. For PHB samples all parameters were the same as inulin with following exceptions: columns set was composed by a pre-column and two columns Phenogel Phenomenex 10^6 and 10^3 and triple point method was based on polystyrene standard provided by Viscotek, with a narrow molecular weight distribution.

2.7. Statistical analysis

The results were statistically analysed through the one-way analysis of variance (ANOVA) test on Minitab® (2013, Minitab Inc.). Experiments were conducted in triplicate and arithmetic means and mean square errors were calculated. Significant differences were determined using the Dunnet's test (significance level: P-value < 0.05).

3. Results and discussions

3.1. Inulin extraction from Cardoon roots

The chemical composition of Cardoon roots (CR) harvested in December 2020 (Winter Cardoon Roots, WCR) and May 2021 (Spring Cardoon Roots, SCR) was determined according to standard NREL protocols for biomass analysis (Sluiter et al., 2008) (Table 1). Both biomasses displayed a high carbohydrate content (about 70 g per 100 g of CR), with glucose being the most abundant sugar, followed by xylose. The greater fructose amount in SCR compared with WCR is ascribable to the highest inulin content accumulated in cardoon roots during the spring (Branca et al., 2022; Raccuia and Melilli, 2010). Lignin amount was comparable (about 33% w/w), regardless of the harvest season.

Both biomasses were subjected to aqueous autoclave-mediated extraction (high temperature and pressure, 120°C 1 bar). The liquors, recovered after the extraction and filtration, were further treated by *i)* direct lyophilization (DL) or *ii)* ethanol precipitation followed by lyophilization (EL). Inulin content was determined for each sample and results compared in Table 2.

Results show that when the extraction liquors are freeze-dried, the amount of recovered extract is independent of starting biomass, resulting into about 25 g of extract per 100 g of roots. Nevertheless, the SCR

Table 1
Compositional analysis of cardoon roots harvested in winter (WCR) and spring (SCR), before (Starting biomass) and after (Residual biomass) the inulin extraction protocol. Residual content refers to data normalized by biomass weight loss (21% for WCR and 27.4% for SCR).

	Starting biomass (% w/w)	Residual biomass (% w/w)	Residual content (%)
WCR			
Glucose	39.1 ± 1.2	25.2 ± 1.1	50.9 ± 0.0
Xylose	12.2 ± 1.0	11.6 ± 1.5	75.1 ± 0.1
Fructose	6.8 ± 1.6	1.7 ± 0.8	19.3 ± 0.1
Mannose	2.4 ± 0.6	1.7 ± 1.0	55.2 ± 0.4
Galactose	2.8 ± 0.2	2.9 ± 0.3	83.3 ± 0.1
Arabinose	0.7 ± 0.1	0.5 ± 0.0	52.7 ± 0.1
Lignin	34.1 ± 0.9	30.8 ± 2.3	71.3 ± 0.1
SCR			
Glucose	33.2 ± 1.4	31.0 ± 0.4	67.8 ± 0.0
Xylose	11.6 ± 1.3	14.2 ± 0.7	88.9 ± 0.1
Fructose	13.9 ± 2.7	2.7 ± 0.6	13.8 ± 0.0
Mannose	6.5 ± 0.2	3.8 ± 0.1	42.2 ± 0.0
Galactose	4.9 ± 0.1	3.1 ± 0.0	45.8 ± 0.0
Arabinose	0.6 ± 0.1	0.5 ± 0.0	54.2 ± 0.1
Lignin	33.5 ± 2.3	43.4 ± 8.1	94.1 ± 0.2

Table 2
Comparison of inulin extraction methods in terms of Raw extract (g of extract powder per 100 g of roots); Purity (g of inulin per 100 g of raw extract); Recovery yield (g of inulin per 100 g of roots).

Cardoon roots	Raw extract (% w/w)		Purity (% w/w)		Recovery yield (% w/w)	
	DL	EL	DL	EL	DL	EL
WCR	21 ± 5.1	5.1 ± 0.4	39 ± 5.6	49 ± 3.4	8	2.45
SCR	25 ± 1.1	12 ± 0.7	64 ± 2.7	93 ± 9.5	16	11.2

extract is characterized by a higher inulin content, expressed by a 64% purity with respect to 39% for WCR. As a consequence, an overall higher recovery yield was obtained for cardoon roots harvested in spring (16% *vs* 8%).

A different result is obtained in the case of liquors subjected to ethanol precipitation followed by freeze-drying. The amount of extract recovered from SCR was twice as much as the extract from WCR (12% *vs* 5%). The ethanol precipitation step clearly improved the purity of extract whatever is the initial biomass, reaching up to 93% for SCR. However, despite the increase of extract purity, this additional step resulted into a lower inulin recovery yield compared to sample processed by direct lyophilization. As a fact, it is conceivable that ethanol addition caused the precipitation of only a fraction of oligosaccharides above a certain degree of polymerization (DP) (Ku et al., 2003; Zeaiter et al., 2019).

Regardless the different recovery yields between the two procedures, the data herein obtained are quite lower with respect to an inulin content of ~45% found in literature (Pari et al., 2021), but in the range (7–28%) measured by other authors (Branca et al., 2022) for cardoon roots harvested at different plant growth and differential stages. Although the observed variability in inulin content can be ascribed to the harvest period (Raccuia and Melilli, 2010), cultivation strategies used and environmental conditions (Singh et al., 2022), it is also conceivable that the time between the harvest and drying steps as well as the storage conditions (at 4°C) applied throughout, may have promoted inulin degradation, being the initial water content detrimental for its preservation (Alfano et al., 2022; Pari et al., 2021).

Residual biomasses were characterized in terms of structural carbohydrates after the extraction process: in both cases the almost complete reduction of fructose is achieved, thus implying an effective inulin extraction. Moreover, despite the high temperature and pressure applied to the biomass, a slight weight loss was observed in both cases. As a fact, the lignin content was not substantially affected and most of the cellulosic fraction was recovered (up to 67.8% in SCR) in the residual biomass. In addition, the hemicellulose derived sugars were recovered in different percentages in the two biomasses, probably reflecting their structural diversity depending on the harvest time (Lourenço and Pereira, 2018) (Table 1).

3.2. Characterization of inulin extracts

In order to evaluate differences in terms of seasonality of cardoon roots and extraction method, collected inulin was analyzed through GPC. Chromatograms of analyzed samples are reported in Fig. 1. A multimodal distribution was observed in all samples. For this reason, each chromatogram was evaluated in three different regions identified as: high molecular weight (green R1), medium molecular weight (orange R2) and low molecular weight (blue R3). Weight average molecular weight (Mw), Number average molecular weight (Mn) and Percentage weight fraction (Wt. Fr%) of highlighted regions for each sample are reported in Table 3. At first glance, the sample deriving from SCR and obtained by ethanol precipitation (SEL) showed peaks mainly in medium-low molecular weight regions with a Wt. Fr% of 77 in R2, indicating good purity according with previous analysis. Moreover, among all samples in the same region, it presented the highest Mw and Mn. Inulin from the same spring cardoon root treated with direct lyophilization method (SDL) reported a slightly minor Wt. Fr% and Mw Mn but a doubled Wt. Fr% in low molecular weight region, corresponding to the oligomeric fraction retained in ethanol step for SEL.

Considering winter samples (WEL and WDL), polymer distributions in medium molecular weight region are comparable as Wt. Fr% but WEL has a slightly higher Mw Mn. Curiously, these samples reported a higher Wt. Fr% in high molecular weight distribution region. Comparing the seasons, roots collected in spring contain higher quantity of inulin and oligomeric fraction with respect to winter ones, in agreement with the vegetative period (Raccuia and Melilli, 2010).

To acquire additional data on the composition of the inulin extracts from the different extraction methods, both SDL and SEL were analyzed also by HPLC. In agreement with the estimated purity degree of the extracts, SDL showed a chromatographic profile characterized by more intense and/or a greater number of peaks than the SEL, suggesting the presence of phenolic compounds with low molecular weights (Fig. 2). In particular, the main peak eluted at 11.6 min and detected in higher amounts in the case of SDL, was identified as 3,4-dihydroxybenzoic acid (3,4-DHBA) by comparison of the chromatographic properties with those of reference standards. In addition, LC-MS analysis allowed for the identification of a set of compounds of the SDL extract that includes, besides 3,4-DHBA, several chlorogenic acid isomers (Figure S1) together with vanillic acid, *p*-hydroxybenzoic acid and dicaffeoylquinic acid in lower amount.

3.3. Enzymatic hydrolysis of inulin extracts

Both inulin extracts from SCR (SEL and SDL) were submitted to enzymatic hydrolysis by the inulinases mixture produced by *Penicillium lanosocoeruleum* (*PlaI*), characterized by the presence of both exo and endo-inulinase activities (Corrado et al., 2021a).

Kinetics of fructose release and inulin reduction were monitored (Fig. 3). A preliminary characterization of the two extracts was carried out to quantify their starting content of reducing sugars. SEL was characterized by about 9 g/L of reducing sugars, versus 24 g/L in SDL. When the sugar composition of the two extracts was analyzed, both were found mainly composed of fructose (about 98% in SEL and 86% in SDL), with SDL displaying a more heterogeneous composition (10% glucose, 3% xylose and 1% galactose). Thus, the percentage of fructose release during hydrolysis was calculated by subtracting the starting fructose content.

The release of fructose in SEL sample increases linearly, to reach

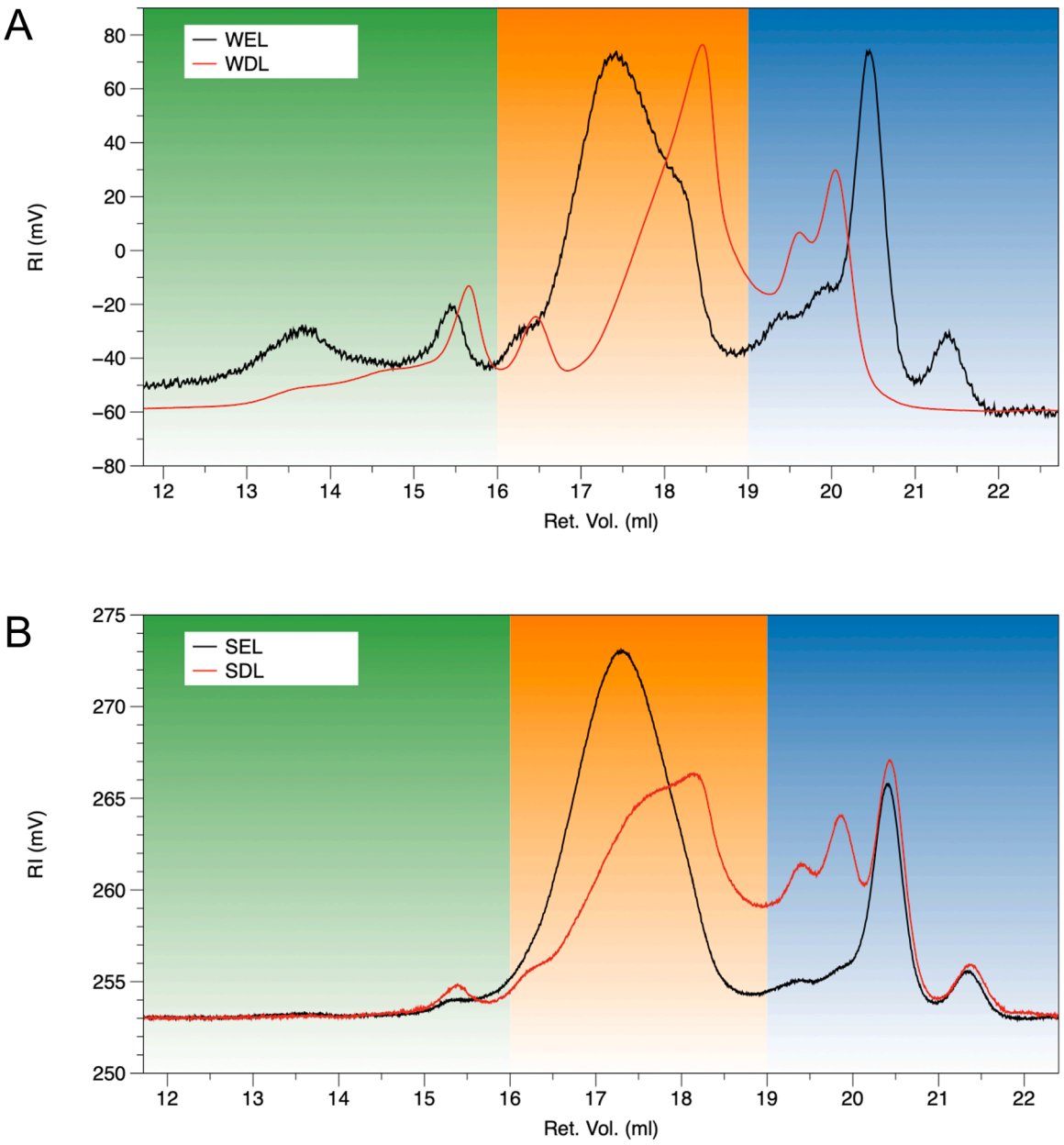


Fig. 1. Overlayed chromatograms at refractive index (RI) of WCR (panel A) and SCR (panel B). Samples obtained by ethanol precipitation (WEL and SEL) are in black, whilst the ones obtained by lyophilization in red (WDL, SDL). The figure is divided in the following regions: high molecular weight (green R1), medium molecular weight (orange R2) and low molecular weight (blue R3).

Table 3
Mw, Mn and Wt. Fr% of inulin samples divided in high molecular weight region (green R1), medium molecular weight region (orange R2) and low molecular weight region (blue R3).

	SEL	SDL	WEL	WDL
Mw R1 (Da)	63704	50776	1.243 e 9	152919
Mn R1 (Da)	53174	15832	41873	49149
Wt. Fr.% R1	2.54	3.11	18.54	23.32
Mw R2 (Da)	8183	2834	5339	3366
Mn R2 (Da)	6333	2019	5121	2837
Wt. Fr.% R2	77.10	56.23	54.13	52.28
Mw R3 (Da)	283	113	395	789
Mn R3 (Da)	179	39	265	378
Wt. Fr.% R3	20.36	40.66	27.34	24.40

Considering the higher recovery yield obtained with SCR with respect to WCR, further work was undertaken focusing on SCR derived extracts.

about 90% in 20 h and settling in a plateau level of about 95% of fructose release at 24 h. Concomitantly, a strong reduction of inulin is achieved in only 2 h, followed by a gradual reduction up to 90% for the remaining reaction time.

The hydrolysis on SDL extract shows a different profile (Fig. 3, panel B): a rapid release of fructose is achieved in the first 4 h, followed by a gradual and slow release over time reaching up to 70% of fructose release after 24 h. Inulin levels decreased sharply at 2 h, keeping constant at 4 h, while reducing down to around 7% after 16 h. The highest fraction of low molecular weight species characterizing the inulin in SDL makes a high concentration of oligosaccharide extremities available for the exo-inulinase activity, thus explaining the steepest fructose release observed in SDL hydrolysis respect to SEL. The apparently low residual inulin content, despite the 70% of fructose release, may be due to errors associated to the assay method, that is affected by the high concentrations of reducing sugars present in the SDL. Furthermore, the different

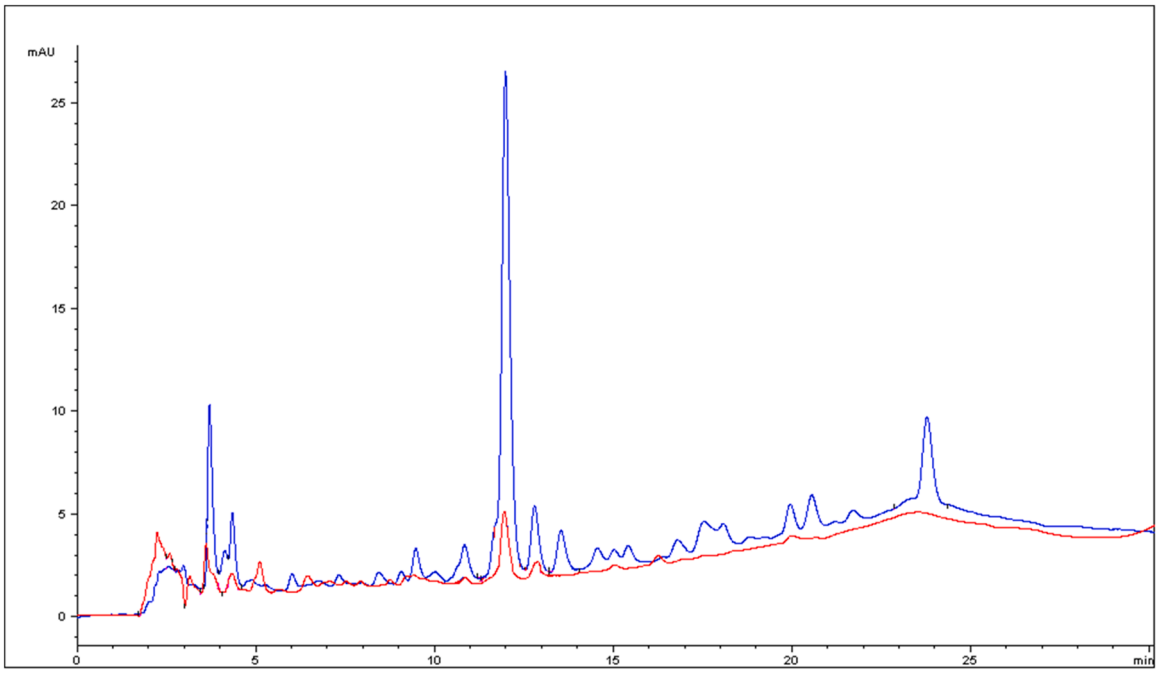


Fig. 2. HPLC profile of SDL (blue trace) and SEL (red trace) extracts.

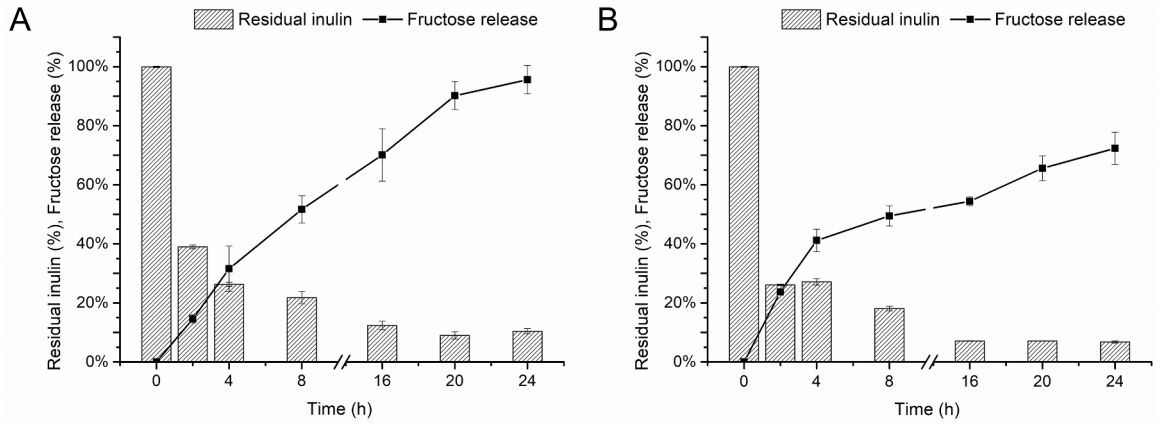


Fig. 3. Kinetics of inulin hydrolysis by *PlaI* on SEL and SDL extracts (panels A and B, respectively). The percentage of fructose release is expressed as g of fructose released at t_i respect to the maximum amount achievable for the complete hydrolysis of the substrate.

trends observed between the SEL and SDL hydrolyses may be a consequence of the diverse purity of the starting extracts (about 64% for SDL *vs* 93% for SEL), affecting the kinetics of both exo- and endo-inulinases present in the inulinase mixture. Noteworthy, the efficiency of *PlaI* hydrolysis on the tested extracts was comparable with the data previously obtained on high purity grade commercial chicory inulin in the same experimental conditions, indicating negligible interference of extract impurities on the enzymatic activity (Corrado et al., 2021a).

Finally, the enzymatic hydrolyses of the two extracts resulted into an effective fructose release, corresponding to a final fructose concentration of about 62 g/L and 43 g/L, respectively for SEL and SDL. Both the hydrolysates were exploited into the following microbial fermentation processes.

3.4. Simultaneous saccharification and fermentation of SEL and SDL extracts

The suitability of the SEL and SDL extracts as carbon source for microbial processes was tested in a simultaneous saccharification and fermentation (SSF) process consisting of inulin hydrolysis catalysed by the inulinase mixture *PlaI*, and a microbial fermentation aimed at PHA production. Two PHA-producers were tested in the process, *B. cepacia* DSM 50181 and *C. necator* DSM 545. The fermentation was carried out using 20 g/L of SEL or SDL extracts (corresponding to an inulin amount of 18.6 g/L and 12.8 g/L respectively) in the presence of 40 U *PlaI*/g extract, according to conditions set in Corrado 2021 (Corrado et al., 2021a). Kinetics of PHA production, residual biomass and reducing sugars were monitored and compared for the two microorganisms (Fig. 4).

When SEL extract was used as C-source, *C. necator* provided the highest polymer accumulation at 72 h, resulting into a Yp/x of 0.32 at 72 h (Fig. 4, panel A). The reducing sugars slightly changed during the fermentation, suggesting a comparable rate of inulin hydrolysis and sugar uptake by the microorganism. At the end of the process, almost all the inulin was hydrolyzed (residual amount about 9%). Compared to *C. necator*, *B. cepacia* displayed a reduced efficiency in PHA production on SEL, reaching a Yp/x of 0.21 at 48 h, despite the almost complete substrate consumption. After 48 h a decrease in polymer amount was

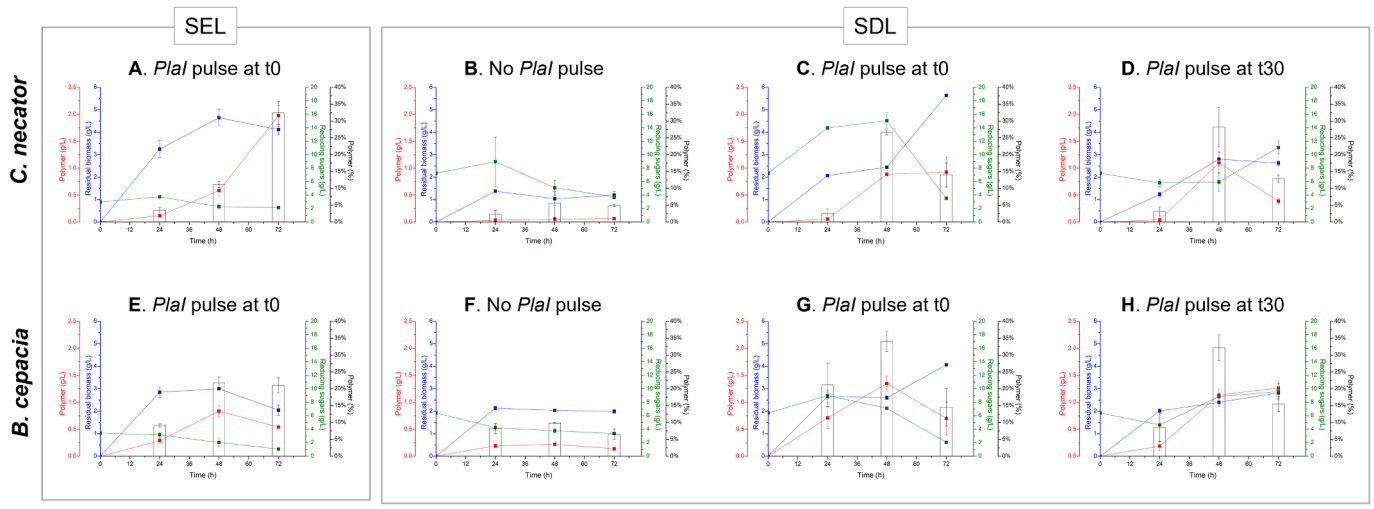


Fig. 4. Kinetic of residual biomass (g/L), polymer production (g/L), reducing sugars (g/L) and polymer accumulation (%), monitored during *C. necator* (A, B, C, D) and *B. cepacia* (E, F, G, H), on: SEL with *PlaI* addition at t0 (respectively A, E); SDL with no *PlaI* addition (respectively B, F); SDL with *PlaI* addition at t0 (respectively C, G); SDL with *PlaI* addition at t30 (respectively D, H).

observed, suggesting its metabolization. Also in this case, the inulin substrate was found consumed after 72 h (3% residual content) (Fig. 4, panel E).

A different scenario occurred when the SSF was carried out on SDL extract (Fig. 4, panels B, C, D, F, G, H). *C. necator* underperformed with respect to SEL in terms of polymer production, with a Yp/x of 0.26, but a lower polymer concentration (about 0.9 g/L at 48 h *vs* 2 g/L for SEL) at 48 h. The trend of residual sugars, that increased in the first phase of the growth and then deeply decreased after 48 h, indicated a slower sugar metabolization with respect to inulin hydrolysis. As a fact, a marked increase in residual biomass was observed only after 48 h. Since the SDL extract displayed a higher content of reducing sugar at t0, it was hypothesized that an excess of sugars (the ones present at t0 and the ones obtained by inulin hydrolysis) may inhibit or slow-down the microbial growth. Thus, the effect of a delayed (at 30 h) addition of *PlaI* was explored (Fig. 4, panel D). However, the postponed sugar availability seemed to not further promote polymer accumulation. As a fact, a comparable Yp/x of 0.28 at 48 h (corresponding to 1.2 g/L of polymer production) was achieved, with a sharp decrease of polymer content at 72 h, despite an increasing concentration of reducing sugars. In both the tested conditions, the residual inulin content was less than 10%. In conclusion, the SEL extract proved to be a better substrate for PHA production by *C. necator* than SDL.

Conversely, the production of PHA by *B. cepacia* seemed to benefit from the use of the SDL extract. In this case, when the *PlaI* mixture was added at t0 (Fig. 4, panel G), up to 1.4 g/L of polymer (Yp/x of 0.34) was obtained at 48 h, followed by a decrease at 72 h, possibly associated with the depletion of carbon source availability and related metabolism of polymer reserves (Keenan et al., 2004). A sharper increase in biomass was observed in the first 24 h, together with a faster decrease in reducing sugars when compared to *C. necator* in the same condition, indicating a better adaptation to the SDL by *B. cepacia*. When *PlaI* was added at 30 h (Fig. 4, panel H), an equivalent polymer concentration was achieved at 48 h with respect to *PlaI* addition at t0, however a slightly increasing trend in both biomass and sugar concentrations was observed at 72 h, indicating the concomitance of the growth and polymer production phases. Whatever was the *PlaI* addition time, about 4% residual inulin was quantified in the culture medium after 72 h.

Comparing the performances of the two strains on the different substrates, it appears that polymer production is more sensitive to the occurrence of proper triggering factors (*i.e.* a reduction in nitrogen availability and/or an excess of the C-source) in *C. necator* 545 than in *B. cepacia*. As a fact, in *C. necator* polymer production: peaked when biomass growth slowed down in SEL at 72 h (possibly indicating a nitrogen limitation); was triggered at 48 h in SDL t0 pulse, when an excess of sugars is available; was limited to a short time frame at 48 h in SDL t30 pulse. On the other hand, *B. cepacia* displayed a growth-related polymer production in all the tested conditions. This is consistent with a recent study, wherein, the related strain *B. cepacia* B1–2 has been hypothesized to have a higher threshold of nitrogen source constraining PHA accumulation, which was responsible of the higher polymer content in N-rich conditions compared to several *Pseudomonas* species (Xu et al., 2022).

In summary, the two microorganisms displayed different performances on the two extracts. Interestingly, when they were cultured in SDL without any *PlaI* addition (Fig. 4, panels B, F), *C. necator* produced up to 0.06 g/L of polymer after 72 h, *vs* 0.22 g/L obtained with *B. cepacia* after 48 h, with the latter displaying a higher biomass yield. Besides the observed response to the high sugar content of SDL at t0, a higher tolerance to the impurities contained in the SDL extract could be displayed by *B. cepacia* (Crooks et al., 2018; Pan et al., 2012a). However, LC-MS analyses of both extracts allowed to rule out the presence of common inhibitors derived from lignocellulose treatment (*e.g.*, furfural, 5-hydroxymethylfurfural, furfuryl alcohol and 2,5-furandicarboxylic acid) as well as levulinic acid. In addition, the lignin-derived aromatic compounds, identified in the SDL extract, were efficiently metabolized by both *C. necator* and *B. cepacia* as evaluated by LC-MS analysis considering the consumption of the aromatic substrates within 72 h fermentation (Figure S2) (Xu et al., 2022).

Although further bioprocess implementation in bioreactor will be required to optimize polymer yields, to the best of our knowledge, this is the first example of valorization of cardoon inulin into polyhydroxyalkanoates. Previously examples reported PHB production using inulin from chicory roots or from Jerusalem artichoke, comparing the performances of different *C. necator* strains in SHF or SSF conditions (Corrado et al., 2021a; Koutinas et al., 2013; Turco et al., 2022). It is worth noting that the Yp/x (around 30%) obtained in this work employing *C. necator* 545 in SSF of SEL extract is comparable with the values obtained for a different *C. necator* strain (428) in batch growth carried out in the same experimental conditions (20 g/L inulin and 40 U/g of inulinase mixture *PlaI*) on chicory inulin (Turco et al., 2022), pointing out the usability of cardoon roots as an alternative source of inulin. Besides, the use of *B. cepacia* for inulin valorization was not explored so far. The results obtained in this work, especially in the case of SDL, confirmed the potential of this strain for the bioconversion of complex feedstocks, as formerly reported for lignin (Xu et al., 2022).

3.5. Characterization of produced polymers

Polymers produced in all the conditions explored above were analyzed by ^{1}H NMR, taking 72 h as reference harvest time. The spectra confirmed the characteristic signals attributable to the PHB homopolymer (Figure S3) for all the analyzed conditions. The same samples were also analyzed by GPC, which confirmed their PHB nature (Fig. 5). As a fact, the refractive index increment (dn/dc), a value characteristic for the polymer/eluent/temperature combination, was comparable for all the samples and consistent with that measured for a commercial PHB (Sigma-Aldrich) (dn/dc 0.0030±0.0004) (Table 4).

GPC analyses also evidenced that *B. cepacia* samples derived from SEL displayed a monomodal distribution (Fig. 5, panel A), while a multimodal distribution was observed for SDL pulse t0 and t30 samples. Among the latter samples, differences in terms of Mn and IV are remarkable indicating differences in polymerization due to pulse feeding.

Regarding *C. necator* samples, all peaks have a multimodal distribution (Fig. 5, panel B). Along these series, a similar trend in terms of feed/molecular weight is observed with respect to *B. cepacia*, but *C. necator* SDL t30 pulse showed a higher Mn and Mw than the corresponding *B. cepacia* sample.

Overall differences in terms of Mn, Mw and IV among SEL and SDL derived samples are ascribable to several factors, such as, the type, activity and levels of PHA synthase and depolymerase, together with feeding mode and substrate availability (Chin et al., 2022; Penloglou et al., 2012). Similarly to what was achieved in this work, a higher polydispersity was observed for PHB produced by *C. necator* 428 in pulsed-batch with respect to simple batch operation mode (Turco et al., 2022). The observed behavior could suggest a different polymerization pathway in which *B. cepacia* starts new polymer chains due to pulse while *C. necator* has the tendency to extend already formed polymer chains, at same reaction time.

4. Conclusions

Cardoon is a promising multi-purpose crop at the forefront of implementing green alternatives to the common fossil-based plastics. In this context, the exploitation of inulin extracted from cardoon roots as a feedstock for polyhydroxyalkanoates production was proved in this work. Two simple procedures for inulin extraction from cardoon roots were tested, resulting into different product grades and molecular weight distribution. The inulin-rich extracts were employed in SSF processes for PHA production by two different microorganisms, *C. necator* and *B. cepacia*. Both the microorganisms proved effective in transforming cardoon inulin into PHA, although displaying different yields depending on the feeding conditions and the type of inulin extract. Interestingly, the differences in polymer molecular weight detected in the explored processes highlighted the peculiarities of the two microorganisms in response to the C-source feeding, in virtue of their different PHA synthetic machineries.

Table 4
Mn, Mw, IV (Intrinsic Viscosity) and dn/dc of analyzed PHB samples extracted from different culture conditions at 72 h from inoculum.

Sample	Mn (Da)	Mw (Da)	IV (dL/g)	dn/dc
***B. cepacia* SEL**	193650	329005	4.026	0.030
***B. cepacia* SDL t0 pulse**	260786	382258	3.669	0.029
***B. cepacia* SDL t30 pulse**	298474	389983	5.202	0.030
***C. necator* SEL**	148523	313242	3.771	0.030
***C. necator* SDL t0 pulse**	227289	385088	4.769	0.030
***C. necator* SDL t30 pulse**	370286	443561	5.623	0.030

Further investigation will be required to evaluate the feasibility of the proposed approach for inulin recovery and subsequent fermentation, assessing the economic impact and the scalability of the extraction steps and considering the effect of the choice of proper microorganism/extract combination on the final polymer yield.

Furthermore, despite the work was focused on inulin valorization, the characterization of the residual biomass after inulin extraction revealed that the applied process did not substantially alter its sugar content, offering the prospect of further valorization of these exhausted roots.

In conclusion, the results add a piece of knowledge in the field of cardoon-based biorefinery, providing the first evidence of root inulin valorization into microbial processes for PHA production.

Funding

This research was funded by Italian Ministero dell'Istruzione dell'Università, CARDIGAN project (CARDoon valorization by InteGrAted biorefinery, Progetti di Ricerca di Interesse Nazionale-Bando 2017).

The work was partly funded by the European Union Next Generation EU PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) within the following project: MICS (Made in Italy – Circular and Sustainable) Extended Partnership (PNRR-MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.3 – D.D. 1551.11–10–2022, PE00000004).

This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

CRediT authorship contribution statement

Giovanni Dal Poggetto: Methodology, Investigation. **Elisabetta Borselleca:** Writing – original draft, Visualization, Methodology, Investigation. **Iolanda Corrado:** Writing – original draft, Visualization, Methodology, Investigation. **Cinzia Pezzella:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Maria Laura Alfieri:** Methodology, Investigation. **Ivana Staiano:** Methodology.

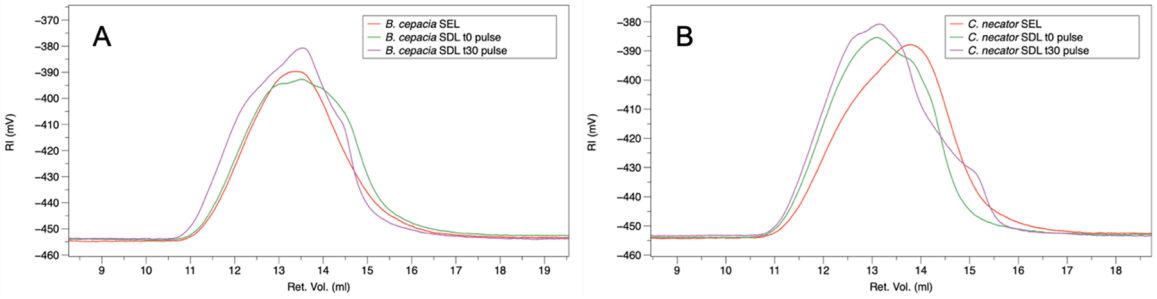


Fig. 5. Overlayed chromatograms at refractive index of *B. cepacia* SEL (red), *B. cepacia* SDL t0 pulse (green) and *B. cepacia* SDL t30 pulse (purple) (panel A); *C. necator* SEL (red), *C. necator* SDL t0 pulse (green) and *C. necator* SDL t30 pulse (purple) (panel B).

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Acknowledgements

We are grateful to Novamont S.p.A. for the kind donation of the cardoon plants. Graphical abstract was created with Biorender.com.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2024.118570.

References

Alfano, V., Stefanoni, W., Latterini, F., Liuzzi, F., De Bari, I., Viola, E., Ciancolini, A., Pari, L., 2022. Inulin content in chipped roots of cardoon stored at different initial moisture contents after six-month storage. Front. Energy Res. 10, 1–9. https://doi.org/10.3389/fenrg.2022.834443.

Borroni, M., Pozzi, C.M., Daniotti, S., Gatto, F., Re, I., 2021. Multi-criteria decision-making approach for nutraceuticals greener applications: the Cynara cardunculus case study. Sustain 13. https://doi.org/10.3390/su132313483.

Branca, F., Argento, S., Paoletti, A.M., Melilli, M.G., 2022. The physiological role of inulin in wild cardoon (Cynara cardunculus L. var. sylvestris Lam.). Agronomy 12, 1–12. https://doi.org/10.3390/agronomy12020290.

Calvo-Flores, F.G., Martin-Martinez, F.J., 2022. Biorefineries: achievements and challenges for a bio-based economy. Front. Chem. 10, 1–23. https://doi.org/10.3389/fchem.2022.973417.

Chin, J.H.C., Samian, M.R., Normi, Y.M., 2022. Characterization of polyhydroxyalkanoate production capacity, composition and weight synthesized by Burkholderia cepacia JC-1 from various carbon sources. Heliyon 8. https://doi.org/10.1016/j.heliyon.2022.e09174.

Ciancolini, A., Alignan, M., Pagnotta, M.A., Vilarem, G., Crinò, P., 2013. Selection of Italian cardoon genotypes as industrial crop for biomass and polyphenol production. Ind. Crops Prod. 51, 145–151. https://doi.org/10.1016/j.indcrop.2013.08.069.

Corrado, I., Cascelli, N., Ntasi, G., Birolo, L., Sannia, G., Pezzella, C., 2021a. Optimization of Inulin Hydrolysis by Penicillium lanosocoeruleum Inulinases and Efficient Conversion Into Polyhydroxyalkanoates. Front. Bioeng. Biotechnol. 9, 1–17. https://doi.org/10.3389/fbioe.2021.616908.

Corrado, I., Petrillo, C., Isticato, R., Casillo, A., Corsaro, M.M., Sannia, G., Pezzella, C., 2021b. The power of two: an artificial microbial consortium for the conversion of inulin into Polyhydroxyalkanoates. Int. J. Biol. Macromol. 189, 494–502. https://doi.org/10.1016/j.ijbiomac.2021.08.123.

Corrado, I., Varriale, S., Pezzella, C., 2023. Microbial Processes for Upcycling Food Wastes Into Sustainable Bioplastics. in F.S. Elsevier. https://doi.org/10.1016/B978-0-12-823960-5.00029-9.

Corrado, I., Vastano, M., Cascelli, N., Sannia, G., Pezzella, C., 2021c. Turning Wastes into Resources: Exploiting Microbial Potential for the Conversion of. Food Wastes into Polyhydroxyalkanoates. https://doi.org/10.1007/978-981-15-9696-4_6.

Cotana, F., Cavalaglio, G., Gelosia, M., Coccia, V., Petrozzi, A., Ingles, D., Pompili, E., 2015. A comparison between SHF and SSSF processes from cardoon for ethanol production. Ind. Crops Prod. 69, 424–432. https://doi.org/10.1016/j.indcrop.2015.02.064.

Crooks, C., Palmer, J.M., Lindner, D.L., 2018. Draft genome sequence of Burkholderia cepacia ATCC 17759, a Polyhydroxybutyrate-Co-Valerate Copolymer-Producing Bacterium. Genome Announc 6. https://doi.org/10.1128/genomeA.00348-18.

Gominho, J., Curt, M.D., Lourenço, A., Fernández, J., Pereira, H., 2018. Cynara cardunculus L. as a biomass and multi-purpose crop: a review of 30 years of research. Biomass-.-. Bioenergy 109, 257–275. https://doi.org/10.1016/j.biombioe.2018.01.001.

Haas, C., Steinwandter, V., De Apodaca, E.D., Madurga, B.M., Smerilli, M., Dietrich, T., Neureiter, M., 2015. Production of PHB from chicory roots - Comparison of three Cupriavidus necator strains. Chem. Biochem. Eng. Q 29, 99–112. https://doi.org/10.15255/CABEQ.2014.2250.

Hossain, M.A., Mushill, L., Rahaman, M.S., Mains, S.M., Vickers, T., Tulaphol, S., Dong, J., Sathitsuksanoh, N., 2022. Upcycling agricultural waste to biodegradable polyhydroxyalkanoates by combined ambient alkaline pretreatment and bacterial fermentation. Ind. Crops Prod. 185, 114867 https://doi.org/10.1016/j.indcrop.2022.114867.

Kalia, V.C., Singh Patel, S.K., Shanmugam, R., Lee, J.K., 2021. Polyhydroxyalkanoates: Trends and advances toward biotechnological applications. Bioresour. Technol. 326, 124737 https://doi.org/10.1016/j.biortech.2021.124737.

Keenan, T.M., Tanenbaum, S.W., Stipanovic, A.J., Nakas, J.P., 2004. Production and characterization of poly-β-hydroxyalkanoate copolymers from Burkholderia cepacia utilizing xylose and levulinic acid. Biotechnol. Prog. 20, 1697–1704. https://doi.org/10.1021/bp049873d.

Koutinas, A.A., Garcia, I.L., Kopsahelis, N., Papanikolaou, S., Webb, C., Villar, M.A., López, J.A., 2013. Production of fermentation feedstock from jerusalem artichoke tubers and its potential for polyhydroxybutyrate synthesis. Waste Biomass-.-. Valoriz. 4, 359–370. https://doi.org/10.1007/s12649-012-9154-2.

Ku, Y., Jansen, O., Oles, C.J., Lazar, E.Z., Rader, J.I., 2003. Precipitation of inulins and oligoglucoses by ethanol and other solvents. Anal., Nutr. Clin. Methods 81, 125–132.

Lourenço, A., Pereira, H., 2018. Compositional Variability of Lignin in Biomass, in: Poletto, M. (Ed.), Lignin. IntechOpen, Rijeka. https://doi.org/10.5772/intechopen.71208.

Mauromicale, G., Pesce, G.R., Curt, M.D., Fernández, J., González, J., Gominho, J., Tabla, R., Roa, I., Portis, E., 2019. Cynara cardunculus as a Multiuse Crop BT - The Globe Artichoke Genome, in: Portis, E., Acquadro, A., Lanteri, S. (Eds.),. Springer International Publishing, Cham, pp. 65–98. https://doi.org/10.1007/978-3-030-20012-1_4.

Mirpoor, S.F., Patanè, G.T., Corrado, I., Giosafatto, C.V.L., Ginestra, G., Nostro, A., Foti, A., Gucciardi, P.G., Mandalari, G., Barreca, D., Gervasi, T., Pezzella, C., 2023. Functionalization of Polyhydroxyalkanoates (PHA)-Based Bioplastic with Phloretin for Active Food Packaging: Characterization of Its Mechanical, Antioxidant, and Antimicrobial Activities. Int. J. Mol. Sci. 24 https://doi.org/10.3390/ijms241411628.

Mirpoor, S.F., Varriale, S., Porta, R., Naviglio, D., Spennato, M., Gardossi, L., Giosafatto, C.V.L., Pezzella, C., 2022. A biorefinery approach for the conversion of Cynara cardunculus biomass to active films. Food Hydrocoll. 122, 107099 https://doi.org/10.1016/j.foodhyd.2021.107099.

Negrulescu, A., Patrulea, V., Mincea, M.M., Ionascu, C., Vlad-Oros, B.A., Ostafe, V., 2012. Adapting the reducing sugars method with dinitrosalicylic acid to microtiter plates and microwave heating. J. Braz. Chem. Soc. 23, 2176–2182. https://doi.org/10.1590/S0103-50532013005000003.

Pan, W., Nomura, C.T., Nakas, J.P., 2012a. Estimation of inhibitory effects of hemicellulosic wood hydrolysate inhibitors on PHA production by Burkholderia cepacia ATCC 17759 using response surface methodology. Bioresour. Technol. 125, 275–282. https://doi.org/10.1016/j.biortech.2012.08.107.

Pan, W., Perrotta, J.A., Stipanovic, A.J., Nomura, C.T., Nakas, J.P., 2012b. Production of polyhydroxyalkanoates by Burkholderia cepacia ATCC 17759 using a detoxified sugar maple hemicellulosic hydrolysate. J. Ind. Microbiol. Biotechnol. 39, 459–469. https://doi.org/10.1007/s10295-011-1040-6.

Pari, L., Alfano, V., Stefanoni, W., Latterini, F., Liuzzi, F., De Bari, I., Valerio, V., Ciancolini, A., 2021. Inulin content in chipped and whole roots of cardoon after six months storage under natural conditions. Sustain 13, 1–11. https://doi.org/10.3390/su13073902.

Penloglou, G., Chatzidoukas, C., Kiparissides, C., 2012. Microbial production of polyhydroxybutyrate with tailor-made properties: an integrated modelling approach and experimental validation. Biotechnol. Adv. 30, 329–337. https://doi.org/10.1016/j.biotechadv.2011.06.021.

Raccuia, S.A., Melilli, M.G., Tringali, S., 2004. GENETIC AND ENVIRONMENTAL INFLUENCE ON INULIN YIELD IN WILD CARDOON (Cynara cardunculus L. VAR. SYLVESTRIS LAM.), in: Acta Horticulturae. International Society for Horticultural Science (ISHS), Leuven, Belgium, pp. 47–53. https://doi.org/10.17660/ActaHortic.2004.660.4.

Raccuia, S.A., Melilli, M.G., 2010. Seasonal dynamics of biomass, inulin, and water-soluble sugars in roots of Cynara cardunculus L. F. Crop. Res. 116, 147–153. https://doi.org/10.1016/j.fcr.2009.12.005.

Singh, R.S., Singh, T., Hassan, M., Larroche, C., 2022. Biofuels from inulin-rich feedstocks: a comprehensive review. Bioresour. Technol. 346, 126606 https://doi.org/10.1016/j.biortech.2021.126606.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2008. Natl. Renew. Energy Lab. Determ. Struct. Carbohydr. lignin Biomass-.-. 17. NREL/TP-510-42618.

Turco, R., Corrado, I., Zannini, D., Gargiulo, L., Di Serio, M., Pezzella, C., Santagata, G., 2022. Upgrading cardoon biomass into Polyhydroxybutyrate based blends: a holistic approach for the synthesis of biopolymers and additives. Bioresour. Technol. 363, 127954 https://doi.org/10.1016/j.biortech.2022.127954.

Turco, R., Santagata, G., Corrado, I., Pezzella, C., Di Serio, M., 2021. In vivo and Post-synthesis Strategies to Enhance the Properties of PHB-Based Materials: a review. Front. Bioeng. Biotechnol. 8 https://doi.org/10.3389/fbioe.2020.619266.

Vergara, P., Ladero, M., García-Ochoa, F., Villar, J.C., 2018. Pre-treatment of corn stover, Cynara cardunculus L. stems and wheat straw by ethanol-water and diluted sulfuric acid: comparison under different energy input conditions. Bioresour. Technol. 270, 449–456. https://doi.org/10.1016/j.biortech.2018.09.058.

Xu, T., Zong, Q.J., Liu, H., Wang, L., Liu, Z.H., Li, B.Z., Yuan, Y.J., 2022. Identifying ligninolytic bacteria for lignin valorization to bioplastics. Bioresour. Technol. 358, 127383 https://doi.org/10.1016/j.biortech.2022.127383.

Zeaiter, Z., Regonesi, M.E., Cavini, S., Labra, M., Sello, G., Di Gennaro, P., 2019. Extraction and characterization of inulin-type fructans from artichoke wastes and their effect on the growth of intestinal bacteria associated with health. Biomed. Res. Int. 2019 https://doi.org/10.1155/2019/1083952.