Renewable Energy 220 (2024) 119655

Contents lists available at ScienceDirect

Renewable Energy

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

# Exploitation of spoilage dates as biomass for the production of bioethanol and polyhydroxyalkanoates

Fathia Madi [a], Ridha Hachicha [a], Jesus Enrique Rodriguez Gamero [b,1], Ameya Pankaj Gupte [b], Nicoletta Gronchi [b], Mansour Haddad [c], Lorenzo Favaro [b,*], Sergio Casella [b], Marina Basaglia [b]

[a] Laboratory of Enzymatic Engineering and Microbiology, National Engineering School of Sfax, B.P.1173-3038, Sfax, Tunisia
[b] Department of Agronomy Food Natural resources Animals and Environment (DAFNAE), Università di Padova, Agripolis, Viale dell'Università 16, 35020, Legnaro, PD, Italy
[c] Laboratory of Arid Lands and Oasiann Cropping, Arid Regions Institute of Gabes, Tunisia

ARTICLE INFO

*Keywords:*
Bioethanol
Polyhydroxyalkanotes
Spoilage date
*Saccharomyces cerevisiae*
*Cupriavidus necator*

ABSTRACT

The exploitation of agri-food wastes is of great importance for environmental and economic reasons. Date wastes are attractive biomasses that could be used as a carbon source for the growth of microorganisms to obtain added-value products. In this work, spoilage date syrup, containing 102.01 and 101.00 g/L of glucose and fructose, respectively, was assessed as a feedstock for the production of bioethanol and polyhydroxyalkanoates (PHAs) by *Saccharomyces cerevisiae* MEL2 and *Cupriavidus necator* DSM 545, respectively. The waste date syrup was first evaluated as a carbon source for microbial growth and resulted to sustain the growth of both strains. 47.95 g/L of ethanol, corresponding to the 93.52% of the theoretical yield, were obtained from the fermentation of date syrup by *S. cerevisiae* MEL2, here adopted as a proficient bioethanol yeast strain. Furthermore, *C. necator* DSM 545, a well-known PHAs-producer, was able to accumulate up to 79.20 % (w/w on dry mass) of PHAs. This study demonstrates that bioethanol and PHAs can be obtained from date wastes, contributing to developing cost-effective exploitation of these residues with economic and environmental advantages.

## 1. Introduction

Global greenhouse gas (GHG) emissions are increasing and without further actions aimed at their reduction, global warming is expected to exceed the temperature by 2 °C above the pre-industrialized levels [1]. Two-thirds of total GHG are produced by fossil fuel consumption, while more than 80% of carbon dioxide emissions are caused by fossil fuels [2] which amounted to 35 billion metric tons of carbon dioxide in 2020 [3]. This is almost a 1 billion metric ton increase from 2019 [4]. The GHG emissions associated with conventional plastics production accounted for 3.8% of total global GHG emissions in 2015 [5].

Fuels and plastics are mainly obtained from petroleum; the decline of fossil reserves and the increase of global warming stimulated researchers to look for new strategies to achieve comparable but environmentally sustainable products [6]. Biofuels and bioplastics recently received considerable attention from both the scientific and industrial communities.

Bioethanol is the most widespread biofuel in the world because of the low atmospheric emissions of GHG during combustion. Furthermore, it can be easily mixed with petrol and, for this reason, could be rapidly integrated into fuel distribution systems [7].

Plastics are employed in many sectors such as agriculture, households, automobiles, packaging, etc., and thus are manufactured in massive amounts. The demand for plastics has increased by 5% every year since 1990 [8]. Unfortunately, these materials are mostly inert to microorganisms and chemical attacks and thus remain intact in the environment for long periods [9]. As a consequence, every year, millions of metric tons of plastics are estimated to reach the oceans [10] and in the last 40 years, plastic waste in the North Pacific Ocean has increased by 100-fold [8]. For these reasons, the search for innovative and eco-friendly materials with characteristics similar to those of fossil plastics is a hallmark and bioplastics such as polyhydroxyalkanoates

* Corresponding author.
*E-mail addresses:* madifathia@hotmail.fr (F. Madi), ridha.hachicha@yahoo.com (R. Hachicha), jesus.rodriguez@uluu.com.au (J.E. Rodriguez Gamero), ameyapankaj.gupte@unipd.it (A.P. Gupte), nicoletta.gronchi@phd.unipd.it (N. Gronchi), manasour.haddad@ira.rnrt.tn (M. Haddad), lorenzo.favaro@unipd.it (L. Favaro), sergio.casella@unipd.it (S. Casella), marina.basaglia@unipd.it (M. Basaglia).
[1] Present address: ULUU, Indian Ocean Marine Research Centre, 86 West Coast Drive, Watermans Bay, Western Australia.

https://doi.org/10.1016/j.renene.2023.119655
Received 26 June 2023; Received in revised form 10 October 2023; Accepted 14 November 2023
Available online 16 November 2023

(PHAs) could become an alternative [11]. Synthesized and accumulated by a wide range of microorganisms, PHAs are natural polyesters of hydroxy alkanoic acids, with physico-chemical and mechanical properties similar to those of synthetic plastics, but they are renewable, biodegradable, and biocompatible with mammals' blood and tissues. Therefore, they can be processed and tailored for interesting applications in medicine, food packaging, or agriculture [12].

Although the substitution of fossil-derived products with bioethanol and PHAs could contribute to the reduction of environmental pollution and carbon dioxide emissions, their utilization may raise concerns and problems. Indeed, bioethanol is currently obtained by *Saccharomyces cerevisiae* fermentation on food feedstocks such as corn, sugarcane, and sugar beet, causing competition between food-for-food and food-for-products [13,14]. Furthermore, the industrial production of PHAs is costly because fermentation broths usually contain expensive highly pure simple sugars such as glucose affecting up to 50% of the total manufacturing costs [15]. Consequently, the large-scale production of PHAs is limited and its utilization is restricted [11,16]. Hence, to broaden bioethanol and bioplastics utilization and increase their industrial sustainability and commercialization, the search for new renewable and cheap carbon sources is critical, and thus further studies are needed. Biomass is the only green source available in nature that offers a direct replacement for fossil fuels [17]. Therefore, to reduce costs, a possible solution could be the utilization of surplus or waste biomass that can be elevated to the role of feedstocks for the biotechnological production of both bioethanol and biopolymers.

Ideally, a waste stream suitable as a feedstock should be easily used by microorganisms as a carbon source [18], possibly in facilities integrated into already existing production lines to diminish transportation costs [19]. In this context, date fruit wastes could be a good alternative to expensive carbon sources in fermentation processes. Dates from palms (*Phoenix dactylifera* L.) are among the major fruits in North Africa and they have been used as food for thousands of years [20]. Worldwide, about 120 million date palm trees are cropped on 800,000 ha in more than 30 countries [21]. Arab countries hold 70% of the 120 million world's date palms and are responsible for 67% of the global date production [22]. In 2021 in Tunisia, date production reached an average of 345,000 tonnes (http://www.fao.org/faostat/en/#home) but more than 30% of this large quantity is constituted by low-quality dates. Indeed, the time from harvesting to processing of the dates and during their storage can lead to excessive softening and fruit spoilage. These "spoilage dates" are not consumed by humans because of fungal contaminations and/or insect infestation or their inadequate texture and poor sensorial quality, lack of marketing and export [23,24]. In most of the developing countries, environmentally sustainable waste disposal is still in the beginning due to limited economic resources and infrastructure. As a result, large amounts of organic wastes are generated and abandoned in the environment or simply landfilled [25]. Thus, large amounts of date wastes are discarded daily by the processing industries causing also environmental problems [26]. Dates flesh has a relatively long time of conservation and reducing sugars (mainly sucrose, glucose, and fructose) are the main components with up to 75% of dry matter [27]. Moreover, dates biomass does not need special treatments to release sugars and could be a potential alternative as cheap feedstock for microbial growth and bioconversion, aimed to substitute expensive carbon sources during any fermentation process [28].

Recently, several authors conducted studies on the possible valorization of date wastes taking into consideration the nutritional value and the chemical composition of the date fruit. By-products obtained from date palm wastes have been suggested for various food and non-food applications. As an example, they could be an excellent and inexpensive source of functional food components such as dietary fiber and phenolic acids or used as an adsorbent in wastewater purification and chemical recovery in the electronic industry [29]. In the area of date cropping, no conversion industry is established to transform the huge amount of date residues into economically useful products such as PHAs and bioethanol. For a sustainable and economically feasible PHAs and bioethanol production, new renewable, elements-rich, and inexpensive carbon feedstocks are required to be explored [11] Thus, the present work was devoted to evaluating the feasibility of waste date extract as a cheap and locally available carbon source to produce both bioethanol and PHAs by the two industrial strains *Saccharomyces cerevisiae* MEL2 and *Cupriavidus necator* DSM 545, here selected as proficient fermenting yeast and PHAs accumulator, respectively.

Although in previous works, date wastes have been utilized in microbial fermentations for the production of organic acids, biofuels, and enzymes [29], to the best of our knowledge, this is the first time that waste dates were evaluated as a feedstock for both bioethanol and PHAs production by two proficient strains and thus this research is relevant to the processing of such low-cost agricultural wastes into biofuel and bioplastics with applications in a wide range of sectors. Moreover, looking for new and environmentally friendly utilization of date wastes well combines with the current concepts of bioeconomy as suggested by the 2030 Agenda for Sustainable Development, adopted by all United Nations Member States in 2015, that provides a shared blueprint for peace and prosperity for people and the planet, suggesting strategies that improve health and education, reduce inequality, and spur economic growth, all tackling climate change and working to preserve our oceans and forests.

## 2. Materials and methods

### 2.1. Raw material and preparation of date syrup

Spoilage dates (dates of poor taste quality) used in this work were a mixture of three varieties, Bouhattam, Kenta, and Lemsi, abundantly present in the region of Gabes (Tunisia). A blend of 10 kg of each variety from 3 different palms, was harvested in the oasis of Gabes during September and October 2017. The mixture was immediately frozen at −20 °C before being processed as described below.

The extraction of date syrup was performed as described by Chniti *et al.* (2014) with some modifications [23]. In short, the fruits were thawed, pitted, crushed with a sharp knife to obtain the pulp, and then lyophilized. Seeds were frozen until analyses were performed. To obtain the syrup, deionized water was added to 300 g dry date pulp to reach a volume of 1 L. The extraction of soluble sugars was carried out on a hot plate at 85 °C for 45 min. The resulting juice was centrifuged at 5500 rpm for 30 min at 4 °C, the supernatant was then pasteurized for 20 min at 75 °C and frozen at −20 °C until utilization.

### 2.2. Bioethanol production by S. cerevisiae MEL2 in date extract

*S. cerevisiae* MEL2, previously isolated in Italy from grape marcs and described as a highly efficient fermenting yeast [30], was adopted to assess the fermentability of date syrup. Inocula were prepared in 200 mL of 6.7 g/L Yeast Nitrogen Base (YNB) without amino acids supplemented with 20 g/L glucose in 500 mL Erlenmeyer flasks and incubated on a rotary shaker (30 °C) at 150 rpm for 24 h. The yeast cells were collected, centrifuged (5400×*g* for 5 min), washed twice with sterile distilled water, and used to inoculate 50 mL YNB without amino acids containing date syrup to obtain a sugar concentration of 51 and 49 g/L of glucose and fructose, respectively. To prevent bacterial contamination, ampicillin and streptomycin were added to a concentration of 100 and 75 mg/L, respectively. To simulate the simple sugars content available in the date syrup, a reference fermentation in the same broth supplemented with 100 g/L glucose was also performed. The initial optical density ($OD_{600\ nm}$) was 1.0. The fermentations were conducted in 60 mL glass serum bottles as described in Favaro *et al.* [31]. The bottles were incubated at 30 °C on a rotary shaker (200 rpm). Yeast growth was periodically monitored by measuring the $OD_{600nm}$. Samples were also analyzed for sugars (glucose, fructose), ethanol, and glycerol by High-Performance Liquid Chromatography (HPLC) as previously

described [32]. The ethanol yield, (g of ethanol/g of used glucose equivalent) was determined as reported in paragraph 2.4 Chemical analyses.

### 2.3. Culturing C. necator DSM 545 with date extract as a carbon source and PHAs production

*C. necator* DSM 545 was provided by DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany. The strain was maintained in glycerol stock at −70 °C and used in this work as PHAs producer. To evaluate the ability to develop in date syrup, *C. necator* DSM 545 was inoculated into 250 mL flasks containing 150 mL of DSMZ 81 medium [33] supplemented with the date syrup as a carbon source to have a concentration of glucose and fructose in the medium equal to 15.5 g/L and 14.5 g/L respectively. The initial optical density at 600 nm ($OD_{600nm}$) was 0.05; the flasks were incubated at 30 °C on a rotating shaker at 250 rpm. For an assessment of the cell growth, samples were withdrawn and $OD_{600nm}$ was monitored by a spectrophotometer (Spectronic® Genesys™ 2 PC). In parallel, reference experiments were performed using glucose at 30.0 g/L as equivalent simple sugars available in the date syrup experiments.

To evaluate the ability of *C. necator* DSM 545 to accumulate PHAs, two production strategies, named *one-step* and *two-steps*, were adopted as described below.

In the case of the *one-step*, bacterial pre-cultures, obtained overnight in DSMZ 81 broth supplemented with 30 g/L glucose, were used to inoculate 250 mL flasks containing 150 mL of the same medium formulated with 1 g/L $NH_4Cl$ and date syrup as a carbon source to reach a concentration of glucose and fructose of 15.5 g/L and 14.5 g/L, respectively. The initial $OD_{600nm}$ was 0.05. After 144 h of aerobic growth at 30 °C under shaking (150 rpm), the cells were harvested by centrifugation at 4 °C (4000×*g* for 10 min), washed twice with sterile distilled water, frozen for 24 h at −80 °C and then lyophilized (Freeze Dryer Modulyo, Edwards). After freeze-drying, cell dry mass (CDM) of samples was then measured and PHAs quantified as described in paragraph 2.4 Chemical analyses.

In the *two-steps* procedure, *C. necator* DSM 545 was firstly grown in DSMZ 81 medium for 72 h under shaking at 150 rpm, as described above for the *one step*. Successively, cells were aseptically centrifuged, residual sugars were analyzed in the supernatant and the cell pellet was washed with sterile water. To promote PHAs accumulation, the cells were then transferred into the same broth used for biomass production containing only one-third of the nitrogen and incubated at 30 °C for additional 72 h. At the end of the second step, cells were recovered and CDW, PHAs, and residual sugars were measured as described below.

All experiments were performed in triplicate and standard deviation was reported.

### 2.4. Chemical analyses

In date seeds, pulp, and syrup moisture was determined by drying in an oven at 100 °C to constant weight; cellulose, hemicellulose, lignin, and protein analyses were performed as described in Basaglia *et al.* (2021) [14]. In lyophilized date pulp, syrup as well as in fermentation experiments, the concentrations of sucrose, glucose, and fructose together with ethanol and glycerol when required, were quantified by HPLC as previously described [34]. Samples were filtered through 0.22 μm cellulose acetate membrane and analyzed through a Shimadzu Nexera HPLC system, equipped with a RID-10A refractive index detector (Shimadzu, Kyoto, Japan). The chromatographic separations were carried out using a Phenomenex Rezex ROA-Organic Acid H+ (8%) column (300 mm × 7.8 mm). The column temperature was set at 65 °C and the analysis was executed at a flow rate of 0.6 mL/min using isocratic elution, with 5 mM $H_2SO_4$ as a mobile phase. Analytes were identified by comparing their retention times and the concentrations were calculated using calibration curves of the corresponding external standard.

Quantification of PHAs in lyophilized cells obtained from the *one step* and the *two steps* mode was performed by Gas Chromatographic (GC) analysis as described by Abbondanzi *et al.* [35] and detailed in Favaro *et al.* [36]. A ThermoFinnigan Trace GC, equipped with FID detector and AT-WAX column (30m × 0.25mmx 0.25 μm) was used for the analyses. The gas carrier was helium at a flow rate of 1.2 mL/min and a split/splitless injector with a split ratio of 1:30 was set at 250 °C; the FID temperature was 270 °C and the oven was set at 150 °C. 3-hydroxybutyric acid (Sigma–Aldrich, Italy) and a P(3HB-co-3HV) copolymer (Biopol™; Imperial Chemical Industries, Great Britain) were used as standard. The content of PHAs is reported as % of PHAs on cell dry matter (CDM).

The ethanol yield, $Y_{EtOH/S}$, (g of ethanol/g of utilized glucose equivalent) was determined considering the amount of glucose/fructose consumed during the fermentation and compared to the maximum theoretical yield of 0.51 g of ethanol/g of consumed glucose equivalent.

Statistical analyses were assessed using the Graphpad Prism 5 package (Graphpad Software, Inc., San Diego, California). Descriptive statistics, mean values, and standard deviations were calculated. Data were analyzed also by two ways factorial ANOVA (Analysis Of Variance) with Duncan test.

## 3. Results and discussion

### 3.1. Chemical composition of spoilage dates and dates syrup

The average chemical composition of the evaluated date waste is reported in Table 1. Dry matter of date pulp and seeds was 64.63 ± 1.34 and 83.19 ± 2.60 % of wet weight respectively. Regarding date seed, cellulose and hemicellulose were the main components with low content in terms of ash and protein, and no free sugars have been detected. This is in accordance with other reports dealing with date by-products characterization and valorization [37,38]. However, date seeds are often discarded as waste since they require complex and costly pre-treatments to convert their polysaccharides into simple sugars and fatty acids to be used by microbial strains and converted into valuable products [39]. Therefore, their biotechnological exploitation potential is quite limited. On the contrary, this work focused on date pulp as a promising substrate for the co-production of PHAs and bioethanol.

As expected, the pulp had a low content of ash, hemicellulose, cellulose and lignin (Table 1). Reducing sugars were predominant (25.85 and 25.20 % of the date pulp for glucose and fructose, respectively). Overall, these data agree with those reported for Arab Emirates date fruits [40,41] while sugar content in the date pulp was lower than those described for other Tunisian dates [27,42]. The difference in the chemical compositions could be due to the varieties of dates, different ripening stages, or local agricultural conditions. In accordance with data reported by Salah and colleagues, the high content of reducing sugars in flesh is probably related to the date cultivars used in this work [43].

Free sugars are indeed readily available in date pulp and have been extracted with high efficiency using hot water. The shares of sugars found in syrup approximately reflect their contents in date pulp reported in Table 1: after 45 min of extraction, a date syrup containing 102.01

**Table 1**
Chemical compositions of spoilage date wastes.

| | Date pulp | Date seeds |
|---|---|---|
| Glucose (% DM) | 35.25 ± 0.25 | – |
| Fructose (% DM) | 34.33 ± 0.38 | – |
| Ash (% DM) | 2.23 ± 0.01 | 1.40 ± 0.08 |
| Cellulose (% DM) | 2.82 ± 0.09 | 45.45 ± 1.07 |
| Hemicellulose (% DM) | 3.14 ± 0.10 | 22.07 ± 0.85 |
| Lignin (% DM) | 2.57 ± 0.10 | 3.74 ± 0.41 |
| Raw proteins (% DM) | 3.60 ± 0.03 | 6.20 ± 0.50 |

DM: dry matter; Results are the means of three replicates and, when relevant, the standard deviations are reported.

and 101.00 g/L of glucose and fructose, respectively, was indeed obtained. This corresponds to an overall extraction efficiency of 97.2% since the theoretical total amount of free sugars potentially obtainable from date pulp was 208.86 g/L (105.75 and 103.11 g/L, of glucose and fructose, respectively).

### 3.2. Bioethanol production by S. cerevisiae MEL2 in dates syrup

Fig. 1 reports the growth of *S. cerevisiae* MEL2 yeast in date syrup (Fig. 1A) and in glucose (Fig. 1B), together with sugar consumption, ethanol, and glycerol production.

Ethanol production started immediately within the first hours of fermentation in both substrates. With date syrup, *S. cerevisiae* MEL2 completely metabolized all fructose as well as glucose (Fig. 1A) within the first 24 h of incubation; ethanol concentration increased rapidly reaching a steady-state approximately 30 h after inoculation with a maximum value of 47.95 g/L at 46 h.

$Y_{EtOH/S}$ is the ethanol yield per gram of consumed substrate calculated on the highest ethanol production; Results are the means of three replicates and, when relevant, the standard deviations are reported.

When pure glucose was used as a carbon source and its concentration was similar to the total amount of sugars used in date fermentation, the maximum ethanol (47.00 g/L), was statistically comparable to that of date syrup (47.95 g/L) obtained between 30 and 46 h (Fig. 1 and Table 2). In both the fermentation with pure glucose and date syrup, 100 % of sugars were consumed by yeast; the maximum ethanol yield (% of the theoretical) and, the ethanol yield/consumed carbon (g ethanol/g glucose) were 88.23% and 0.45 g/g, respectively, both significantly lower ($p \leq 0.05$) if compared with those obtained with date syrup (93.52% and 0.48 g/g) (Table 2).

The high conversion values obtained with date syrup by *S. cerevisiae*

**Table 2**
Consumed sugars, ethanol, and glycerol yields by *S. cerevisiae* MEL2 in glucose and date extract.

| Parameter | Growth in glucose | Growth in date syrup |
|---|---|---|
| Added sugars concentration (g/L) | 105.14 | 100.54 |
| Consumed sugars (%) | 100.00 | 100.00 |
| Highest Ethanol (g/L) | 47.00 ± 0.64 | 47.95 ± 2.32 |
| Glycerol (g/L) | 3.31 ± 0.06 | 5.81 ± 0.4 |
| $Y_{EtOH/S}$ | 0.45 | 0.48 |
| % theoretical yield | 88.23 | 93.52 |

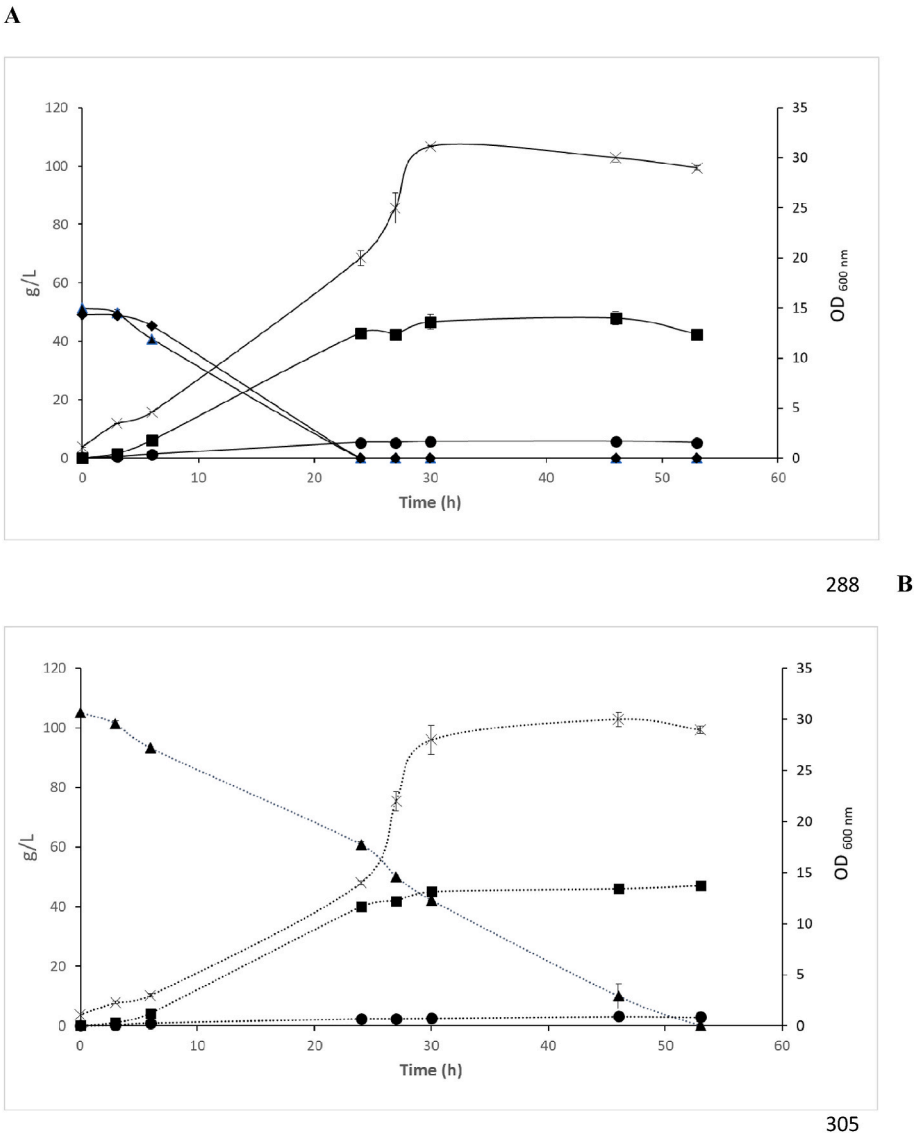


**Fig. 1.** Growth (X), glucose (▲) and fructose (♦) consumption, ethanol (■), and glycerol (●) production by *S. cerevisiae* MEL2 in dates syrup (A, solid line) and glucose benchmark broth (B dashed line). Results are the means of three replicates and the standard deviations are reported.

MEL2 are consistent with the results of Favaro and colleagues reporting for the same yeast strain an outstanding glucose-to-ethanol conversion efficiency of 0.49 g/g, corresponding to the 96% of the theoretical yield using unfiltered wheat bran hydrolysates as carbon source [30]. These results are in agreement with those reported with date extract by Chniti *et al.* and Sulieman and coworkers that found ethanol yields around 94 and 91% of the theoretical values, respectively, with other *S. cerevisiae* strains [23,44].

During alcoholic fermentation, in addition to the ethanol, glycerol was also synthesized by yeast and a significant increase ($p \leq 0.01$) was observed in the case of the data syrup fermentation. Glycerol can indeed balance the external osmotic pressure [23] and equilibrate the intracellular redox balance by converting excess NADH, generated during biomass formation, to $NAD^+$. Overall, both ethanol and glycerol levels produced by the selected yeast strain should be considered of high promise if compared to those available in the recent related literature. Much lower ethanol yields (from 37 to 73% of the theoretical) were indeed recently reviewed [38]. The selection of highly fermenting yeast, *S. cerevisiae* Fm17, proved to be very effective in this study and calls for specific research efforts to improve the overall ethanol yields from date waste fermentation.

### 3.3. Culturing of C. necator DSM 545 on date syrup

In view of the possible industrial exploitation of spoilage dates, the growth of *C. necator* DSM 545 was assessed in media containing the dates syrup or comparable amounts of glucose as a carbon source. While on glucose this strain reached the stationary phase in 60–70 h, on date syrup it was attained in only 40–45 h (Fig. 2).

Thus, date extract seems to support bacterial development even better than pure sugar, possibly due to additional nutrients or important growth factors contained in this complex substrate, although the juices of several fruits such as those of black currant [45], pomegranate [46] or cranberry [47] have been reported as strong inhibitors of bacterial and fungal growth. Date fruits of *Phoenix dactylifera* L., though containing large amounts of nutrients such as sugars, proteins, and vitamins, have also phenolics in large extent with a strong antioxidant activity potentially lowering microbial development [37]. For instance, date extract proved inhibitory on pathogenic yeast such as *Candida* [48], while date syrup was reported to be bacteriostatic to both *Escherichia coli* and *Staphylococcus aureus* [49]. Other authors found an antibacterial activity of *Phoenix dactylifera* L. leaf and pit extracts against Gram-negative and Gram-positive pathogenic bacteria [50]. On the contrary, the date syrup used in this work allowed the microbial growth of industrial strains such as *C. necator* DSM 545. These results therefore indicate that this substrate, rich in easily fermentable sugars, can be an optimal potential feedstock in any bioprocess technology involving this microorganism.

### 3.4. Accumulation of PHAs by C. necator DSM 545 from date syrup

*C. necator* DSM 545 showed the ability to efficiently accumulate PHAs from date syrup as a carbon source, following both the *one-step* and the *two-steps* culturing procedures (Table 3).

At the end of the *one step* process, the strain produced a significant amount of biomass (1.28 g/L) and 0.93 g/L of PHAs, corresponding to 73.20% of the CDM. These results are comparable or even superior to those obtained with *C. necator* with other agro-industrial substrates. For example, Brojanigo and colleagues [51], reported PHAs values of 44% of CDM with enzyme-treated broken rice, while Ramos *et al.* obtained a

**Table 3**
Cell dry mass, sugars consumption, and PHAs accumulation by *C. necator* DSM 545 in media containing date syrup as a carbon source, by the *one-step* and the *two-steps* procedures. The percentage of consumption is reported in brackets.

| | Initial concentration | *One step* | *Two steps* | |
|---|---|---|---|---|
| | | 144 h | 72h | 144h |
| Glucose (g/L) | 15.50 | 0.95 ± 0.21 | 4.70 ± 0.85 | 1.50 ± 1.06 |
| Consumed glucose (g/L) | | 14.55 (93.87%) | 10.80 (67.90%) | 14.00 (90.00%) |
| Fructose concentration (g/L) | 14.50 | 8.65 ± 3.75 | 10.50 ± 0.07 | 7.30 ± 0.42 |
| Consumed fructose (g/L) | | 5.85 (40.34%) | 4.0 (30.00%) | 7.2 (49.60%) |
| CDM (g/L) | | 1.28 ± 0.05 | 0.84 ± 0.04 | 1.70 ± 0.09 |
| PHAs(3HB% of CDM) | | 73.20 ± 4.67 | 67.40 ± 0.55 | 79.20 ± 1.13 |
| PHAs Concentration (g/L) | | 0.93 ± 0.10 | 0.57 ± 0.05 | 1.35 ± 0.09 |
| PHAs Yield[a] (g/g) | | 0.049 | | 0.037 |

CDM: Cell dry mass.
[a] PHAs (g) per consumed sugars (g); Results are the means of three replicates and, when relevant, the standard deviations are reported.

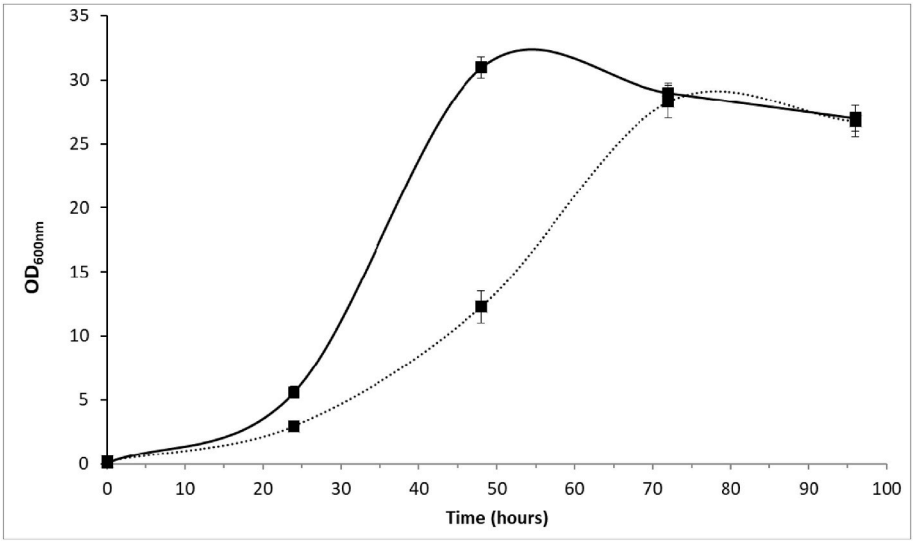


**Fig. 2.** Growth of *C. necator* DSM 545 on media containing dates syrup (solid line) or glucose (dashed line) as a carbon source. Results are the means of three replicates and the standard deviations are reported.

percentage of 58.60% of CDM with alkaline pretreated cocoa husk [52]. On the other hand, PHAs percentage of 67.9% and a superior PHAs yield of 7.4 g/L of PHAs was found with red apple extract [53]. Overall, date syrup was revealed to be a suitable feedstock for the growth as well as the PHAs production from *C. necator*.

Through the *one step* mode, 20.40 g of sugars (14.50 g of glucose and 5.85 g of fructose) were metabolized with a PHAs yield of 0.049.

When the *two steps* process was applied, after 72 h only 67.90% of glucose and 30.00% of fructose were consumed. Biomass (0.84 g/L) was recovered, sugars were added again and the culture was incubated for additional 72 h. After 144 h, total dry mass doubled and reached 1.70 g/L with a PHAs content of 79.20 % of CDW; both cell dry biomass and PHAs concentration (1.35 g/L) were significantly higher ($p < 0.05$) as compared to those obtained in the *one step* mode (+32 and + 44%, respectively). Nevertheless, in the *two steps* mode, 36 g/L of sugars (10.8 g/L of glucose and 4 g/L of fructose in the growth step and 14 g/L of glucose and 7.3 g/L of fructose in the accumulation step) were consumed with a PHAs yield of 0.037 g/g (g of PHA/g of consumed sugar).

Between the two sugars present in date syrup, fructose, and glucose, the latter seems to be preferentially used as a carbon source as more than 90% of glucose and less than 50% of fructose were consumed in both *one step* and *two steps* after 144 h.

In both fermentation modes, large amounts of residual sugars remained unutilized by *C. necator* DSM 545 in the spent media, namely 9.60 g/L (0.95 g of glucose and 8.65 g of fructose) in the *one-step* and 24.00 g/L (6.2 g of glucose and 17.8 g of fructose) in the *two steps* (Table 3). These quantities correspond to around 32 and 40% of the total sugars applied in the two processes (30 g/L in the *one-step* and 60 g/L in the *two-steps*). Thus, although the *two-step* system seems to perform better in terms of total PHAs, the polymer yield per gram of consumed sugars is higher with the *one-step* fermentation mode and the residual sugars are less. Consequently, the *one-step* procedure appears more efficient in terms of sugars to PHAs conversion.

Several studies were conducted to obtain PHAs from dates with different microorganisms. For example, a *Bacillus megaterium* strain, isolated from the sludge of a sewage treatment plant, reached in flasks a cell density of 3.3 g/L from date syrup as a carbon source [54]. This strain was able to accumulate up to 1.71 g/L of PHB corresponding to 52% of CDM with a PHAs yield of 0.045 g of PHAs per gram of consumed sugar. Better results were obtained in fermenter-optimized conditions using a *Bacillus* strain able to accumulate PHAs up to 5.85 g/L corresponding to 70.5 % of CDM and a PHAs yield of 0.45 [55].

The non-pathogenic gram-negative soil bacterium *C. necator* represents the model organism in the contest of PHAs production; moreover, it can be easily genetically modified [15,56], and could be used in industrial PHAs production processes [57,58]. *C. necator* DSM 545 revealed to be a powerful PHAs-accumulating microorganism that may be useful for the cost-effective production of the biopolymer not only from glucose but also from by-producs rich in starch and/or sugarsfeedstocks. As an example, Brojanigo *et al.* [59] reported for the first time an engineered *C. necator* DSM 545 able to produce, in a Consolidated Bioprocessing context [60], high PHAs levels (up to 5.92 g/L). Costa *et al.* found for the same microorganism, high yields of 3.8 of PHB with melon extracts, and lower yields of around 0.5 g/L of PHB for other fruit or vegetable extracts such as tomatoes or pears [53]. Instead, in optimized conditions from pretreated banana fronds extract and by *C. necator* H16, Low and colleagues reported PHB concentrations of 1.35 g/L, similar to those obtained in this work [61].

To the best of our knowledge, no studies have been conducted using *C. necator* DSM 545 and date waste streams as substrate. The overall PHAs amounts attained in this study with *C. necator* DSM 545 and date syrup are consistent with data previously obtained with other bacteria; furthermore, they were achieved in flasks and the optimization of media composition and growth conditions in a fermenter could strongly improve PHAs yield.

## 4. Conclusion

The present study demonstrates that spoilage date is an alternative, low-cost carbon source to be used for both bioethanol and PHAs production without any intense physical or chemical treatment. Due to their composition, the date palm waste stream is an ideal substrate for bioprocesses aimed to obtain a wide range of products and has enormous potential as raw material for bioprocessing.

Although future research focused on the fine-tuning of fermentation parameters is necessary to improve yields, the outcomes obtained in this work pave the way to future industrial exploitation allowing the development of new bio industries and more efficient waste management. Co-productions of bioethanol and PHAs could be obtained together in the future, thanks to process integration. Even though specific efforts are still needed to improve process efficiency, this paper showed that a detailed characterization of a given waste available in a plant is crucial towards the development of biotechnological routes for its full exploitation. Moreover, this paper highlights that even pasteurization of waste can be an effective way to preserve its valuable components without the use of more energy-intensive methods (i.e. autoclaving) thus improving the economic viability of the process.

Overall, this approach could be of great promise with sustainable economic and social positive benefits where a date waste conversion plant will be established. This will be of great importance mainly in developing countries where circular economy approaches can significantly reduce the dependence on non-renewable resources and improve their occupational level thanks to additional job positions.

## CRediT authorship contribution statement

**Fathia Madi:** Investigation, Formal analysis, Writing – original draft. **Ridha Hachicha:** Resources. **Jesus Enrique Rodriguez Gamero:** Investigation. **Ameya Pankaj Gupte:** Investigation, Formal analysis, Writing – review & editing. **Nicoletta Gronchi:** Investigation, Formal analysis. **Mansour Haddad:** Resources. **Lorenzo Favaro:** Conceptualization, Writing – review & editing, Supervision, Resources. **Sergio Casella:** Writing – review & editing, Resources. **Marina Basaglia:** Visualization, Writing – review & editing, Supervision, Resources.

## 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.

## Acknowledgements

This work was partially supported by the University of Padova with the following research projects BIRD234877/23, DOR2352129, DOR2251254/22; DOR2107797/21, DOR2084579/20, DOR1928058/19, BIRD210708.

## References

[1] X. Zheng, D. Streimikiene, T. Balezentis, A. Mardani, F. Cavallaro, H. Liao, A review of greenhouse gas emission profiles, dynamics, and climate change mitigation efforts across the key climate change players, J. Clean. Prod. 234 (2019) 1113–1133, https://doi.org/10.1016/j.jclepro.2019.06.140.

[2] M. Shao, Z. Han, J. Sun, C. Xiao, S. Zhang, Y. Zhao, A review of multi-criteria decision making applications for renewable energy site selection, Renew. Energy 157 (2020) 377–403, https://doi.org/10.1016/j.renene.2020.04.137.

[3] F. Perera, K. Naedu, Climate change, fossil-fuel pollution, and children's health, N. Engl. J. Med. (2022) 2303–2314, https://doi.org/10.1056/NEJMra2117706.

[4] U.K. Pata, Linking renewable energy, globalization, agriculture, $CO_2$ emissions and ecological footprint in BRIC countries: a sustainability perspective, Renew. Energy 173 (2021) 197–208, https://doi.org/10.1016/j.renene.2021.03.125.

[5] J. An, F. Wu, D. Wang, J. You, Estimated material metabolism and life cycle greenhouse gas emission of major plastics in China: a commercial sector-scale

perspective, Resour. Conserv. Recycl. 180 (2022) 1–10, https://doi.org/10.1016/j.resconrec.2022.106161.
[6] A. Razmjoo, L. Gakenia Kaigutha, M.A. Vaziri Rad, M. Marzband, A. Davarpanah, M. Denai, A Technical analysis investigating energy sustainability utilizing reliable renewable energy sources to reduce $CO_2$ emissions in a high potential area, Renew. Energy 164 (2021) 46–57, https://doi.org/10.1016/j.renene.2020.09.042.
[7] A.P. Gupte, M. Basaglia, S. Casella, L. Favaro, Rice waste streams as a promising source of biofuels: feedstocks, biotechnologies and future perspectives, Renew. Sustain. Energy Rev. 167 (2022) 1–19, https://doi.org/10.1016/j.rser.2022.112673.
[8] B. Kunwar, H.N. Cheng, S.R. Chandrashekaran, B.K. Sharma, Plastics to fuel: a review, Renew. Sustain. Energy Rev. 54 (2016) 421–428, https://doi.org/10.1016/j.rser.2015.10.015.
[9] J. Zalasiewicz, C.N. Waters, J.A. Ivar, P.L. Corcoran, A.D. Barnosky, A. Cearreta, M. Edgeworth, A. Ga, C. Jeandel, R. Leinfelder, J.R. Mcneill, W. Steffen, C. Summerhayes, M. Wagreich, M. Williams, A.P. Wolfe, Y. Yonan, The geological cycle of plastics and their use as a stratigraphic indicator of the Anthropocene, Anthropocene 13 (2016) 4–17, https://doi.org/10.1016/j.ancene.2016.01.002.
[10] M. Egger, L. Quiros, G. Leone, F. Ferrari, C.M. Boerger, M. Tishler, Relative abundance of floating plastic debris and neuston in the eastern North Pacific ocean, Front. Mar. Sci. 8 (2021) 1–13, https://doi.org/10.3389/fmars.2021.626026.
[11] L. Favaro, M. Basaglia, S. Casella, Improving polyhydroxyalkanoate production from inexpensive carbon sources by genetic approaches: a review, Biofuels, Bioprod. Biorefining. (2019) 208–227, https://doi.org/10.1002/bbb.1944.
[12] R. Muthuraj, O. Valerio, T.H. Mekonnen, Recent developments in short- and medium-chain- length Polyhydroxyalkanoates: production, properties, and applications, Int. J. Biol. Macromol. 187 (2021) 422–440, https://doi.org/10.1016/j.ijbiomac.2021.07.143.
[13] S. Niphadkar, P. Bagade, S. Ahmed, Bioethanol production: insight into past, present and future perspectives, Biofuels 9 (2018) 229–238, https://doi.org/10.1080/17597269.2017.1334338.
[14] M. Basaglia, M. D'Ambra, G. Piubello, V. Zanconato, L. Favaro, S. Casella, Agro-food residues and bioethanol potential: a study for a specific area, Processes 9 (2021) 1–14, https://doi.org/10.3390/pr9020344.
[15] J.E. Rodríguez Gamero, S. Brojanigo, M. Basaglia, L. Favaro, S. Casella, Efficient production of polyhydroxybutyrate from slaughterhouse waste using a recombinant strain of *Cupriavidus necator* DSM 545, Sci. Total Environ. 794 (2021) 1–7, https://doi.org/10.1016/j.scitotenv.2021.148754.
[16] X. Zhao, K. Cornish, Y. Vodovotz, Narrowing the gap for bioplastic use in food packaging: an update, Environ. Sci. Technol. 54 (2020) 4712–4732, https://doi.org/10.1021/acs.est.9b03755.
[17] P.A. Østergaard, N. Duic, Y. Noorollahi, S. Kalogirou, Latest progress in Sustainable Development using renewable energy technology, Renew. Energy 162 (2020) 1554–1562, https://doi.org/10.1016/j.renene.2020.09.124.
[18] L. Favaro, L. Cagnin, M. Basaglia, V. Pizzocchero, W.H. van Zyl, S. Casella, Production of bioethanol from multiple waste streams of rice milling, Bioresour. Technol. 244 (2017) 151–159, https://doi.org/10.1016/j.biortech.2017.07.108.
[19] M. Koller, A. Atlić, M. Dias, A. Reiterer, G. Braunegg, Microbial PHA production from waste raw materials BT - plastics from bacteria: natural functions and applications, in: G.G.-Q. Chen (Ed.), Plast. From Bact., Springer Berlin Heidelberg, Berlin, Heidelberg, 2010, pp. 85–119, https://doi.org/10.1007/978-3-642-03287-5_5.
[20] W. Al-Shahib, R.J. Marshall, The fruit of the date palm: its possible use as the best food for the future? Int. J. Food Sci. Nutr. 54 (2003) 247–259, https://doi.org/10.1080/09637480120091982.
[21] F. Aslam, A. Ansari, A. Aman, G. Baloch, G. Nisar, A.H. Baloch, H.U. Rehman, Production of commercially important enzymes from *Bacillus licheniformis* KIBGE-IB3 using date fruit wastes as substrate, J. Genet. Eng. Biotechnol. 18 (2020) 1–7, https://doi.org/10.1186/s43141-020-00060-8.
[22] L.I. El-juhany, Degradation of date palm trees and date production in Arab countries : causes and potential rehabilitation, Aust. J. Basic Appl. Sci. 4 (2014) 3998–4010.
[23] S. Chniti, H. Djelal, M. Hassouna, A. Amrane, Residue of dates from the food industry as a new cheap feedstock for ethanol production, Biomass Bioenergy 69 (2014) 66–70, https://doi.org/10.1016/j.biombioe.2014.07.011.
[24] W. Braide, I. Kanu, U. Oranusi, S. Adeleye, Production of bioethanol from agricultural waste, J. Fund. Appl. Sci. 8 (2016) 372–386, https://doi.org/10.4314/jfas.v8i2.14.
[25] A.S. Nizami, M. Rehan, M. Waqas, M. Naqvi, O.K.M. Ouda, K. Shahzad, R. Miandad, M.Z. Khan, M. Syamsiro, I.M.I. Ismail, D. Pant, Waste biorefineries: enabling circular economies in developing countries, Bioresour. Technol. 241 (2017) 1101–1117, https://doi.org/10.1016/j.biortech.2017.05.097.
[26] M. Chandrasekaran, A.H. Bahkali, Valorization of date palm (*Phoenix dactylifera*) fruit processing by-products and wastes using bioprocess technology – review, Saudi J. Biol. Sci. 20 (2013) 105–120, https://doi.org/10.1016/j.sjbs.2012.12.004.
[27] M. Elleuch, S. Besbes, O. Roiseux, C. Blecker, C. Deroanne, N. Drira, H. Attia, Date flesh : chemical composition and characteristics of the dietary fibre, Food Chem. 111 (2008) 676–682, https://doi.org/10.1016/j.foodchem.2008.04.036.
[28] E.A. El-Nagga, Y.A. Abd El Tawab, Compositional characteristics of date syrup extracted by different methods in some fermented dairy products, Ann. Agric. Sci. 57 (2012) 29–36, https://doi.org/10.1016/j.aoas.2012.03.007.
[29] B. Aydeniz-Güneşer, Valorization of date palm (*Phoenix dactylifera*) wastes and by-products, in: M.F. Ramadan, M.A. Farag (Eds.), Mediterr. Fruits Bio-Wastes, Springer International Publishing, Cham, 2022, pp. 391–402, https://doi.org/10.1007/978-3-030-84436-3_16.
[30] L. Favaro, M. Basaglia, W.H. Van Zyl, S. Casella, Using an efficient fermenting yeast enhances ethanol production from unfiltered wheat bran hydrolysates, Appl. Energy 102 (2013) 170–178, https://doi.org/10.1016/j.apenergy.2012.05.059.
[31] L. Favaro, M.J. Viktor, S.H. Rose, M. Viljoen-Bloom, W.H. van Zyl, M. Basaglia, L. Cagnin, S. Casella, Consolidated bioprocessing of starchy substrates into ethanol by industrial *Saccharomyces cerevisiae* strains secreting fungal amylases, Biotechnol. Bioeng. 112 (2015) 1751–1760, https://doi.org/10.1002/bit.25591.
[32] N. Gronchi, L. Favaro, L. Cagnin, S. Brojanigo, V. Pizzocchero, M. Basaglia, S. Casella, Novel yeast strains for the efficient saccharification and fermentation of starchy by-products to bioethanol, Energies 12 (2019) 1–13, https://doi.org/10.3390/en12040714.
[33] J.E. Rodríguez Gamero, L. Favaro, V. Pizzocchero, G. Lomolino, M. Basaglia, S. Casella, Nuclease expression in efficient polyhydroxyalkanoates-producing bacteria could yield cost reduction during downstream processing, Bioresour. Technol. 261 (2018) 176–181, https://doi.org/10.1016/j.biortech.2018.04.021.
[34] L. Cagnin, L. Favaro, N. Gronchi, S.H. Rose, M. Basaglia, W.H. van Zyl, S. Casella, Comparing laboratory and industrial yeast platforms for the direct conversion of cellobiose into ethanol under simulated industrial conditions, FEMS Yeast Res. 19 (2019) 1–13, https://doi.org/10.1093/femsyr/foz018.
[35] F. Abbondanzi, G. Biscaro, G. Carvalho, L. Favaro, P. Lemos, M. Paglione, C. Samorì, C. Torri, Fast method for the determination of short-chain-length polyhydroxyalkanoates (scl-PHAs) in bacterial samples by in Vial-Thermolysis (IVT), Nat. Biotechnol. 39 (2017) 29–35, https://doi.org/10.1016/j.nbt.2017.05.012.
[36] L. Favaro, M. Basaglia, J.E.G. Rodriguez, A. Morelli, O. Ibraheem, V. Pizzocchero, S. Casella, Bacterial production of PHAs from lipid-rich by-products, Appl. Food Biotechnol. 6 (2019) 45–52, https://doi.org/10.22037/afb.v6i1.22246.
[37] M. Al-farsi, C. Alasalvar, M. Al-abid, K. Al-shoaily, M. Al-Amry, F. Al-Rawahy, Compositional and functional characteristics of dates, syrups, and their by-products, Food Chem. 104 (2007) 943–947, https://doi.org/10.1016/j.foodchem.2006.12.051.
[38] A. Taghizadeh-alisaraei, A. Motevali, B. Ghobadian, Ethanol production from date wastes : adapted technologies, challenges, and global potential, Renew. Energy 143 (2019) 1094–1110, https://doi.org/10.1016/j.renene.2019.05.048.
[39] A.J. Jadhav, C.R. Holkar, A.D. Goswami, A.B. Pandit, D.V. Pnjari, Acoustic cavitation as a novel approach for extraction of oil from waste date seeds, ACS Sustain. Chem. Eng. 4 (2016) 4256–4563, https://doi.org/10.1021/acssuschemeng.6b00753.
[40] B. Ismail, I. Haffar, R. Baalbaki, Y. Mechref, J. Henry, Physico-chemical characteristics and total quality of five date varieties grown in the United Arab Emirates, Int. J. Food Sci. Technol. 41 (2006) 919–926, https://doi.org/10.1111/j.1365-2621.2005.01143.x.
[41] H.M. Habib, W.H. Ibrahim, Nutritional quality of 18 date fruit varieties, Int. J. Food Sci. Nutr. 62 (2011) 544–551, https://doi.org/10.3109/09637486.2011.558073.
[42] T. Rihab, B. Maachia Sihem, S. Marianne, S. Ali, N. Ahmed, M. Mars, Chemical fruit composition of Tunisian date palm 'Deglet Nour' collected at maturation from four different oases in Djerid region, J. Food Technol. 17 (2019) 1–10, https://doi.org/10.36478/jftech.2019.1.10.
[43] Mohamed Ben Salah, Food value of soft dates cultivated in Tunisian coastal oases, J. Life Sci. 10 (2015) 234–241, https://doi.org/10.17265/1934-7391/2015.05.007.
[44] A.K. Sulieman, M.H. Gaily, M.A. Zeinelabdeen, M.D. Putra, A.E. Abasaeed, Production of bioethanol fuel from low-grade-date extract, Int. J. Chem. Eng. Appl. 4 (2013) 140–143, https://doi.org/10.7763/ijcea.2013.v4.280.
[45] J. Krisch, L. Galgóczy, M. Tölgyesì, T. Papp, C. Vágvölgyi, Effect of fruit juices and pomace extracts on the growth of Gram-positive and Gram-negative bacteria, Acta Biol. Szeged. 52 (2008) 267–270.
[46] S. Vaithiyanathan, B.M. Naveena, M. Muthukumar, P.S. Girish, N. Kondaiah, Effect of dipping in pomegranate (*Punica granatum*) fruit juice phenolic solution on the shelf life of chicken meat under refrigerated storage (4°C), Meat Sci. 88 (2011) 409–414, https://doi.org/10.1016/j.meatsci.2011.01.019.
[47] J.D. Dubreuil, Fruit extracts to control pathogenic Escherichia coli: a sweet solution, Heliyon. 6 (2020) 1–7. https://doi.org/10.1016/j.heliyon.2020.e03410.
[48] Z.A. Shraideh, K.H. Abu-Elteen, A.K.J. Sallal, Ultrastructural effects of date extract on *Candida albicans*, Mycopathologia 142 (1998) 119–123, https://doi.org/10.1023/A:1006901019786.
[49] H. Taleb, S.E. Maddocks, R.K. Morris, A.D. Kanekanian, The antibacterial activity of date syrup polyphenols against *S. aureus* and *E. coli*, Front. Microbiol. 7 (2016) 1–9, https://doi.org/10.3389/fmicb.2016.00198.
[50] Kahkashan Perveen, Antibacterial activity of *Phoenix dactylifera* L. leaf and pit extracts against selected Gram negative and Gram positive pathogenic bacteria, J. Med. Plants Res. 6 (2012) 296–300, https://doi.org/10.5897/jmpr11.1380.
[51] S. Brojanigo, E. Parro, T. Cazzorla, L. Favaro, M. Basaglia, S. Casella, Conversion of starchy waste streams into polyhydroxyalkanoates using *Cupriavidus necator* DSM 545, Polymers 12 (2020) 1–12, https://doi.org/10.3390/polym12071496.
[52] L.H. Ramos, M. Cisneros-Yupanqui, D.V. Santisteban Soto, A. Lante, L. Favaro, S. Casella, M. Basaglia, Exploitation of cocoa pod residues for the production of antioxidants, polyhydroxyalkanoates, and ethanol, Fermentation 9 (2023) 1–18, https://doi.org/10.3390/fermentation9090843.
[53] P. Costa, M. Basaglia, S. Casella, L. Favaro, Polyhydroxyalkanoate production from fruit and vegetable waste processing, Polymers 14 (2022) 1–12, https://doi.org/10.3390/polym14245529.
[54] S. Omar, A. Rayes, A. Eqaab, I. Voß, A. Steinbüchel, Optimization of cell growth and poly(3-hydroxybutyrate) accumulation on date syrup by a *Bacillus megaterium*

strain, Biotechnol. Lett. 23 (2001) 1119–1123, https://doi.org/10.1023/A:1010559800535.
[55] M.A. Khiyami, S.M. Al-fadul, A.H. Bahkali, Polyhydroxyalkanoates production via *Bacillus* PCS biofilm and date palm syrup, J. Med. Plants Res. 5 (2014) 3312–3320.
[56] S. Povolo, P. Toffano, M. Basaglia, S. Casella, Bioresource Technology Polyhydroxyalkanoates production by engineered *Cupriavidus necator* from waste material containing lactose, Bioresour. Technol. 101 (2010) 7902–7907, https://doi.org/10.1016/j.biortech.2010.05.029.
[57] M. Koller, A review on established and emerging fermentation schemes for microbial production of polyhydroxyalkanoate (PHA) biopolyesters, Fermentation 4 (2018) 1–30, https://doi.org/10.3390/fermentation4020030.
[58] F. Reinecke, A. Steinbüchel, *Ralstonia eutropha* strain H16 as model organism for PHA metabolism and for biotechnological production of technically interesting biopolymers, J. Mol. Microbiol. Biotechnol. 16 (2008) 91–108, https://doi.org/10.1159/000142897.
[59] S. Brojanigo, N. Gronchi, T. Cazzorla, T.S. Wong, M. Basaglia, L. Favaro, S. Casella, Engineering *Cupriavidus necator* DSM 545 for the one-step conversion of starchy waste into polyhydroxyalkanoates, Bioresour. Technol. 347 (2022) 1–7, https://doi.org/10.1016/j.biortech.2021.126383.
[60] L. Favaro, M. Basaglia, M. Saayman, S. Rose, W.H. van Zyl, S. Casella, Engineering amylolytic yeasts for industrial bioethanol production, Chem. Eng. Trans. 20 (2010) 97–102.
[61] T.J. Low, S. Mohammad, K. Sudesh, S. Baidurah, Utilization of banana (*Musa* sp.) fronds extract as an alternative carbon source for poly(3-hydroxybutyrate) production by *Cupriavidus necator* H16, Biocatal. Agric. Biotechnol. 34 (2021) 1–7, https://doi.org/10.1016/j.bcab.2021.102048.