Valorisation of fruit wastes for the production of poly(3-hydroxybutyrate) and value-added co-products

Olga Psaki, Dimitrios Ladakis, Maria Alexandri, Apostolis Koutinas
Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, 11855, Athens, Greece.

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Presenting author email: olgapsaki@outlook.com

1. Introduction

The excessive use of plastics in the last decades has caused major environmental issues leading to the need of some new alternatives in order to replace the conventional petroleum-derived plastics with bio-based ones. Poly(3-hydroxybutyrate) (PHB) is a biodegradable thermoplastic that could replace petroleum-derived plastics, such as polypropylene. The main reason than hinders industrial production of PHB is the high production cost, mainly the substrates used (glucose, vegetable oils) in the fermentation process. For this reason, research has focused on the evaluation of inexpensive substrates, such as lignocellulosic residues and food wastes, which do not compete with food production (Follonier et al., 2014, Kennedy et al 1999). In this regard, fruit pomaces (composed of fruit skins, pulp, stalks and seeds) could be utilized not only as fermentation feedstock, but also for the production of other value-added products. The high free sugar and structural polysaccharide content renders this raw material ideal for bioconversion. Moreover, fruits are rich in pectins that could be separated as a value-added co-product with many current and potential market segments. Phenolic compounds extracted from fruits find applications in food and cosmetic industries owing to their antioxidant and antimicrobial properties (Follonier et al., 2014, Bustamante et al., 2008).

In this work, fruit wastes were valorized through their refining into value-added fractions and the production of fermentation feedstock for PHB production. Three different fruits were studied (pears, apples and peaches) and their composition was determined. Free sugars were initially extracted to be utilized for PHB production. The separation of pectin-rich extracts and phenolic compounds was investigated. PHB production by the bacterial strain *Burkholderia sacchari* DSM 17165 has been evaluated in different ratios of carbon to nitrogen using the free sugars from fruits and commercial sugars.

2. Material and Methods

Fruit wastes (apple, pear, peach) were collected from a local market in Athens and macerated using a kitchen blender. Free sugars were extracted under different solid-to-liquid ratios (1:10, 1:15, 1:20) at 40ºC for 2 h. Phenolic compounds were extracted by ultrasound-assisted extraction at solid-to-liquid ratios of 1:10, 1:20, 1:30 (w/v) using 70% acidified aqueous ethanol. Extraction of pectin-rich extracts was carried using different acids (hydrochloric acid, nitric acid and citric acid) at pH 2 in temperatures ranging from 75-87 ºC.

PHB production was carried out using the bacterial strain *Burkholderia sacchari* DSM 17165 under aerobic conditions. The effect of different carbon sources (sucrose, glucose, fructose, free sugars) together with the effect of carbon-to-nitrogen (C/N) ratio was studied in 500 mL shake flasks with 100 mL working volume. All fermentations were supplemented with a mineral medium having the following composition (per L): $Na_2HPO_4 \cdot 2H_2O$, 4.5 g; KH_2PO_4, 1.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; and a trace elements solution, 1.0 mL, and incubated at 30^0C and at 180 rpm. The trace element solution has the following composition (per L): $FeSO_4 \cdot 7H_20$, 10 g; $ZnSO_4 \cdot 7H_2O$, 2.25 g; $CuSO_4 \cdot 5H_2O$, 1 g; $MnS0_4 \cdot 4H_2O$, 0.5 g; $CaCl_2 \cdot 2H_2O$, 2 g; $Na_2B_4O_7 \cdot 10H_2O$, 0.23 g; $(NH_4)_6Mo_7O_{24}$, 0.1 g; 35% HC1 10 mL. Precultures were prepared using the same mineral composition together with (per L): $(NH_4)_2SO_4$, 1.0 g yeast extract, 1.0 g and a trace elements solution, 1.0 mL, with 10 g/L of glucose and incubated for 12 h at 30^0C and at 180 rpm. Organic (yeast extract) and inorganic nitrogen (ammonium sulfate) sources were evaluated in different C/N ratios (160, 80, 40, 20, 10) in order to evaluate microbial growth and production of PHB under nitrogen limitation.

Fed-batch bioreactor fermentations were also carried out using the free sugars from fruits. Fermentation was carried out in a 3.6 L bioreactor (LabFors 5) with 1.5 L working volume at 30 ºC. The phosphorus concentration was the limiting factor in order to trigger polymer accumulation. The initial medium composition for the fed-batch culture was (per L): $(NH_4)_2SO_4$, 4.0 g; KH_2PO_4, 3.0 g; citric acid, 1.7 g; EDTA, 40 mg; trace elements solution, 10 mL; $MgSO_4 \cdot 7H_2O$, 1.2 g. The pH was controlled automatically at 6.8 with 28% NH_4OH which was also used to avoid the depletion of nitrogen in the fermentation broth and 2 M HCl solution. The aeration rate used was 3.6 L air/min. The dissolved oxygen set-point was 20% saturation and a cascade of agitation speed of 1200 - 900 rpm was used.

Sugars concentrations were determined by HPLC-RID and PHB content was determined by acid propanolysis according to Riss et al. (1985) and analyzed by Gas Chromatography. Free amino nitrogen and inorganic phosphorus concentrations were determined spectrophotometrically following the protocols of Lie et al., (1973) and Harland and Harland (1980).

3. Results and discussion

Free sugars were extracted using dH_2O under three different solid-liquid ratios from all the selected fruit wastes. Among the ratios tested, the 1:20 ratio appeared to be the most effective, with 94.14% yield extraction for apple, 90.80% for pear and 83.97% for peach. The main sugars were fructose (38-68%), glucose (20-36%) and sucrose (2.5-18%). Subsequently, phenolic compounds were extracted under different ratios of acidified aqueous ethanol. The highest value of total phenolic content was achieved at the ratio of 1:30 (w/v), where the total phenolic content in apple was approximately 900 mg GAE/100g (dw) and for pear was 600 mg GAE/100g (dw). Subsequently, the extraction of pectin-rich extract was evaluated. The highest yield of pectin-rich extract separation was achieved when HCl was applied in apples (12.90%), pear (13.20%) and peaches (9.63%).

PHB accumulation by *B. sacchari* was initially investigated using commercial sugars under different C/N ratios. When yeast extract was used as nitrogen source, the lower C/N ratios resulted in increased polymer accumulation. The strain showed preference in glucose as growth and PHB accumulation presented higher values, in comparison to the ones achieved with fructose and sucrose. The highest PHB content was observed at C/N 20, with values of 67%, 65% and 60% when glucose, fructose and sucrose were used as carbon sources, respectively. Biomass concentrations were around 13 g/L in all cases. Increasing the C/N ratio to 160 led to reduced PHB accumulation in all sugars.

Since lower C/N were the most efficient for PHB accumulation, the C/N ratios of 1:20 and 1:10 were applied when ammonium sulfate was evaluated as nitrogen source. The accumulation of PHB at C/N 10 ratio was 36%, 37% and 40% when fructose, sucrose and free sugars were used as substrates, while biomass concentrations of 9 g/L, 8 g/L and 9 g/L were reached, respectively.

Free sugars from fruits were utilized for PHB production in fed-batch bioreactor cultures leading to more than 100 g/L total dry weight with a PHB content of more than 50% (w/w).

4. Conclusions

Fruit wastes from open markets has been holistically valorized via fractionation of phenolic-rich extracts and pectin-rich extracts combined with bioconversion of free sugars into PHB. The use of yeast extract le to higher biomass and PHB concentrations. Fed-batch bioreactor cultures with free sugars and ammonium sulphate gave promising results on PHB production.

5. References

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