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

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Objectives

- **Introduction**
  - ✓ Poly(3-hydroxybutyrate) (P3HB) structure and synthesis
  - ✓ P(3HB) applications
  - ✓ Food wastes in Europe
- **Characterization and pretreatment of raw materials**
  - ✓ Chemical composition
  - ✓ Extraction and determination of free sugars
  - ✓ Extraction and Determination of Total phenolic content with the method Folin-Ciocalteu
  - ✓ Extraction of pectin and determination of galacturonic acid
- **P(3HB) production by *Burkholderia sacchari***
  - ✓ Evaluation of nitrogen source in shake flasks fermentations
  - ✓ Fed-batch fermentation in bioreactor with phosphorous limitation strategy
- **Conclusions**

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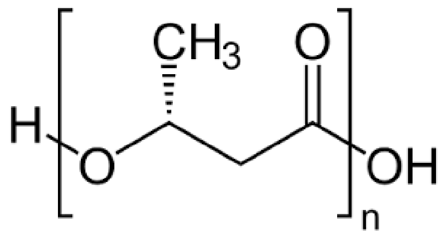
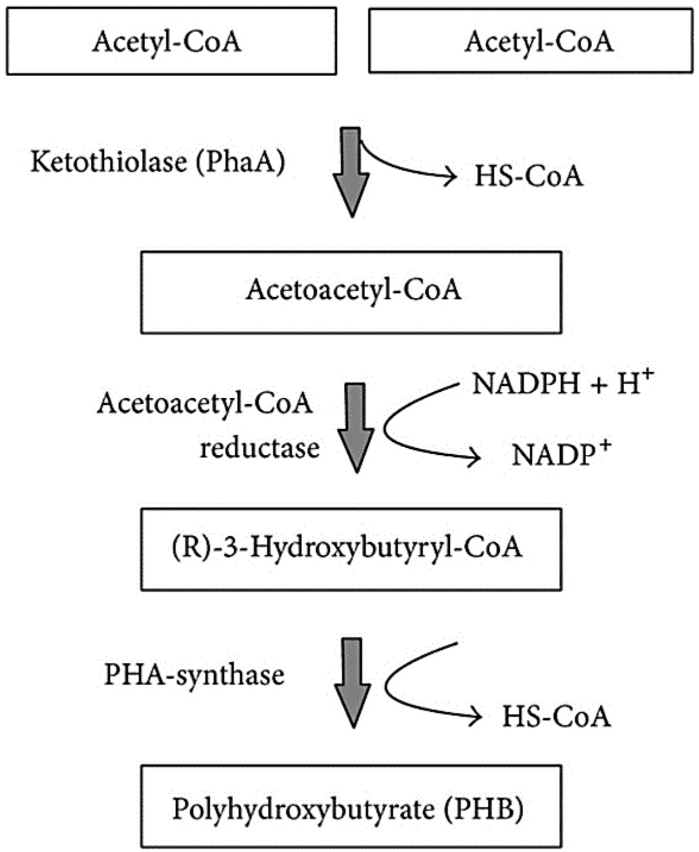
Poly(3-hydroxybutyrate) (P3HB) structure and synthesis

- Member of polyhydroxyalkanoates, polymer of polyesters
- Accumulated in intracellular granules by Gram + and – microorganisms
- Poly-beta-hydroxybutyrate (PHB), the polymeric ester of four D(-)-3 hydroxybutyrate
- In an excess of carbon source and a nutritional limitation factor (N, P, O)





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P(3HB) applications

Area	Application
Agricultural	Encapsulation of fertilizers
Biomedical	Sutures, supports of tissue cultures for implants, surgical implants, part of bones and replanted veins, engineering of heart valves and pins
Packaging	Bags, bottles and food packaging
Pharmacological	Encapsulation of medicines for controlled release
Industrial	Recovery of oligomers and monomers for new use in the synthesis of polymers

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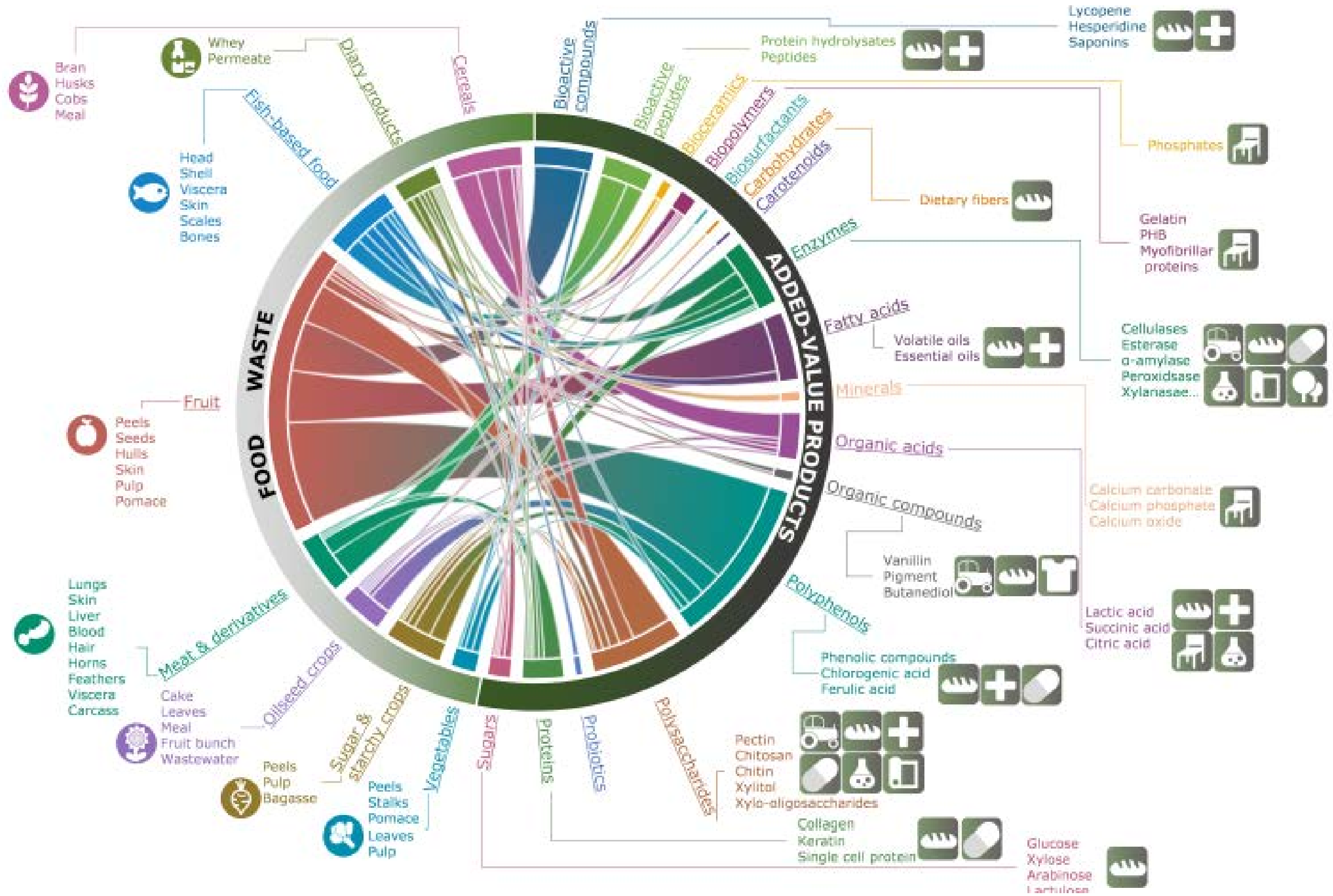
Food wastes in Europe



- It is estimated that around 129 million tonnes of food are wasted annually in EU
- Fresh fruit and vegetables contribute to almost 45% of the food waste generated
- EU households generate 35.3 kg of fresh fruit and vegetable waste per person per year

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Chemical composition



Composition (% Dry basis)	APPLE		PEAR		PEACH	
	Present Study	Literature	Present Study	Literature	Present Study	Literature
Moisture content (%)	84.4	81.0-86.6	83.5	81.0-85.0	87.2	84.7-87
Protein content	2.6	1.6-3.7	3.2	1.3-3.8	3.5	4.20
Free sugars	71.7	44.7-65.0	75	42.0-75	72.1	46.9-76.5
Lipid content	0.8	1.3±0.1	0.3	1.3-3.8	0.3	0.2-0.7
Ash content	2.7	2.2±0.7	2.4	0.2-5.5	4.1	2.6-3.1
Total Phenolics content (g GAE/100g Dry weight)	0.9	0.2-0.13	0.6	0.25-0.85	0.47	0.2-0.4
Pectin content	10	7.8-13.2	8.4	3.1-14.2	8.9	2-10
Cellulose	5.3	3.4-8.81	4	4.6-7.9	3.9	1.33-3.58
Lignin	1.2	0.6-2.98	2.8	1.3-2.7	2.5	0.73-4.13
Hemicellulose	4.8	0.6-5.44	3.3	2.5-3.0	4.1	1.6-3.9

- ✓ Rich in pectins
- ✓ Contains high amounts of free sugars
- ✓ Main sugars: Glucose, Fructose, Sucrose

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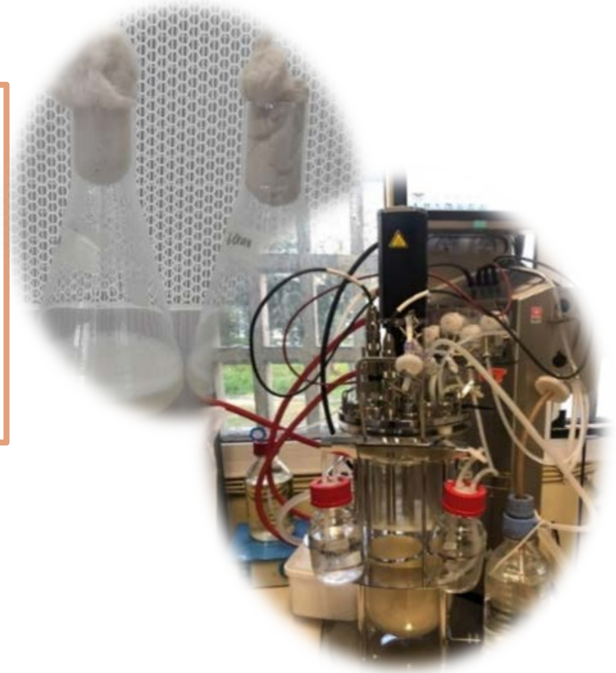
Experimental process

FREE SUGARS

- Evaluation of nitrogen source in shake flasks fermentations
- Fed-batch fermentation in bioreactor with phosphorous limitation strategy



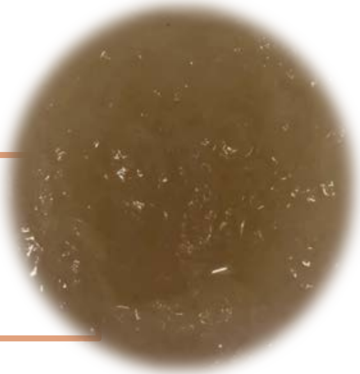
Solid to liquid ratio: 1:20 (w/v) H_20

Residual solids

Residual solids

Extraction of phenolics compounds

Evaluation of organic acids for pectin extraction



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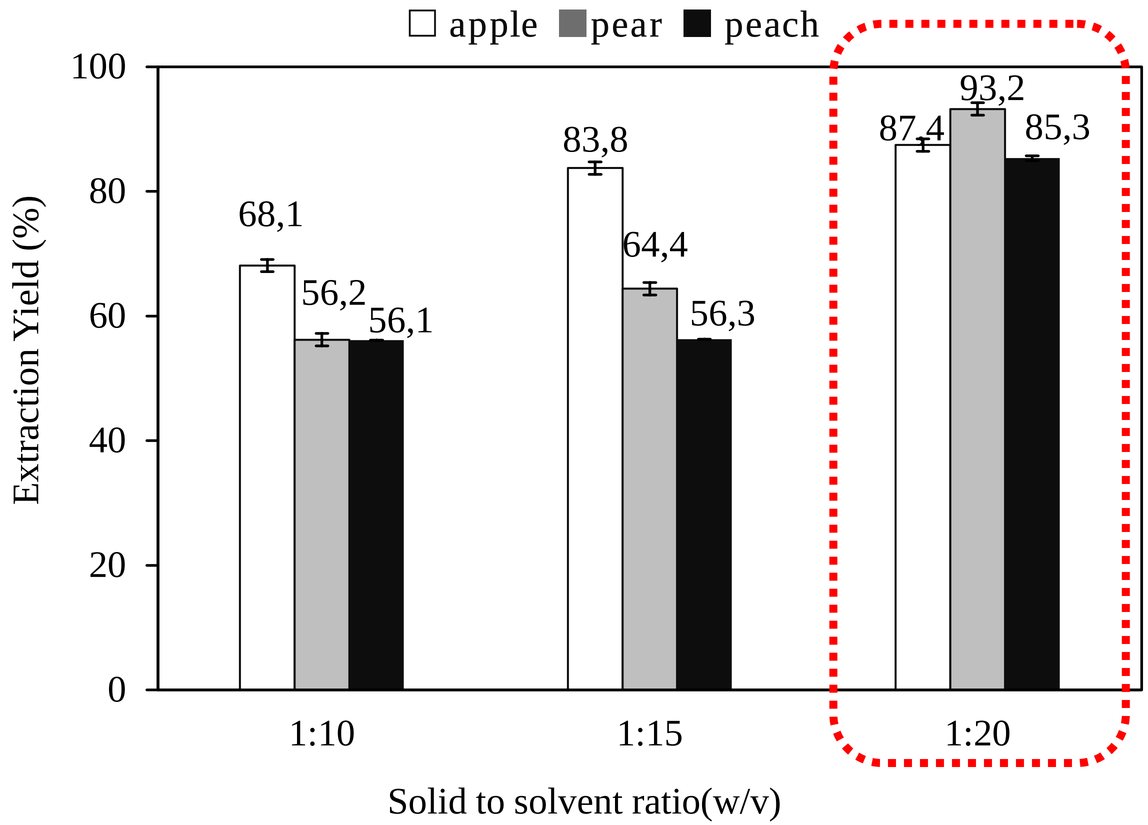
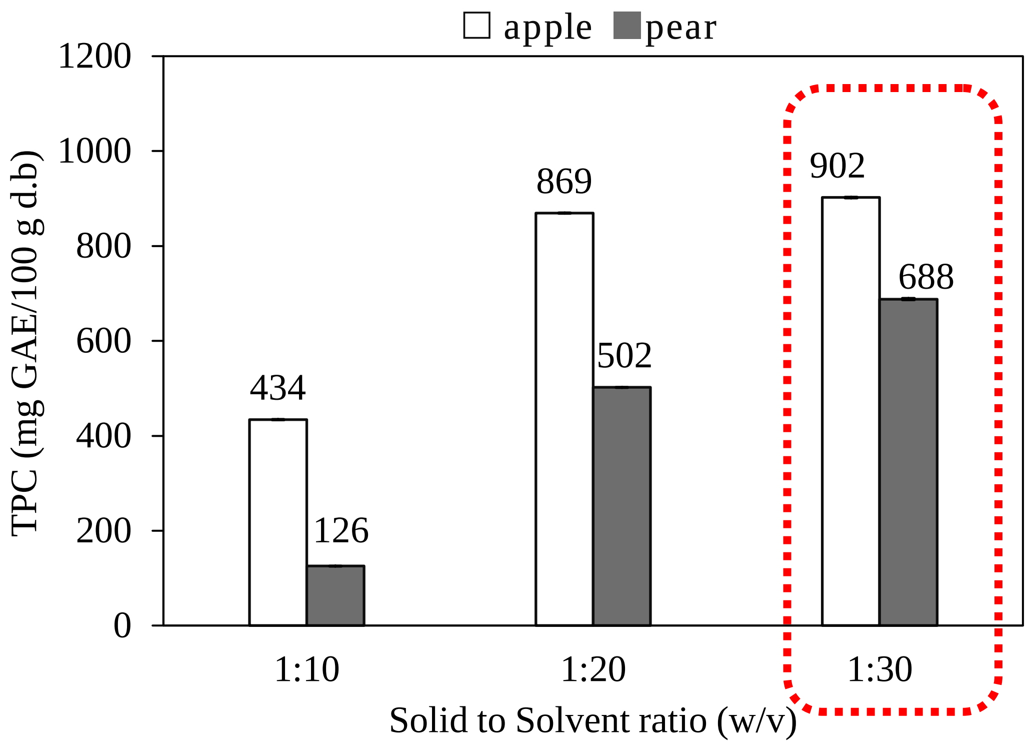
Extraction of free-water soluble sugars

Conditions	
Solid to Solvent ratios (w/v)	1:10, 1:15, 1:20
Duration	2 hours
Temperature	45^0 C



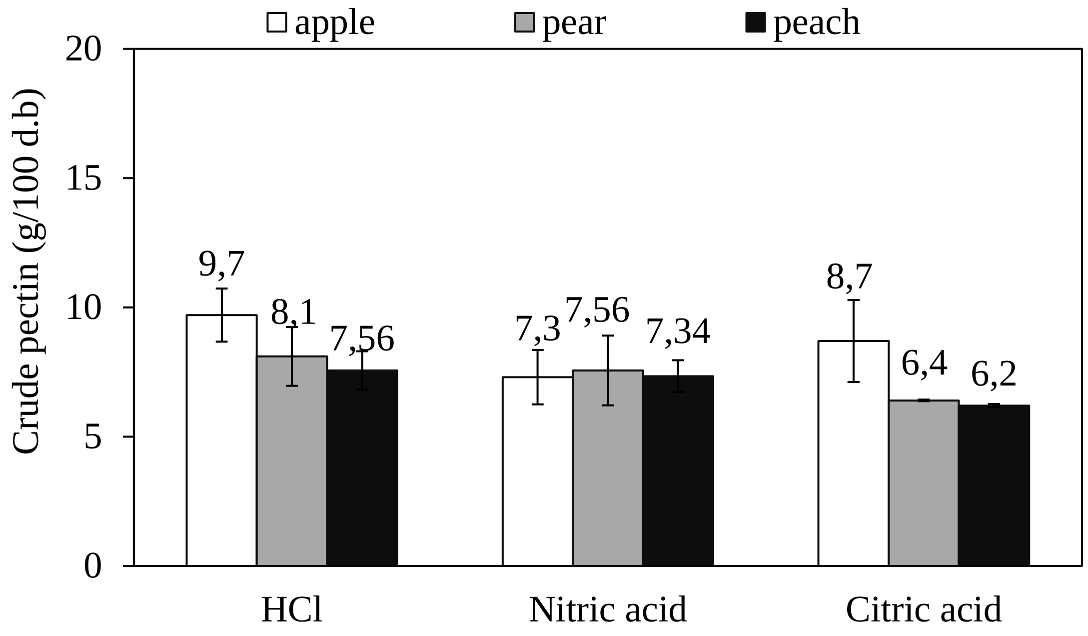
Extraction of total phenolic content

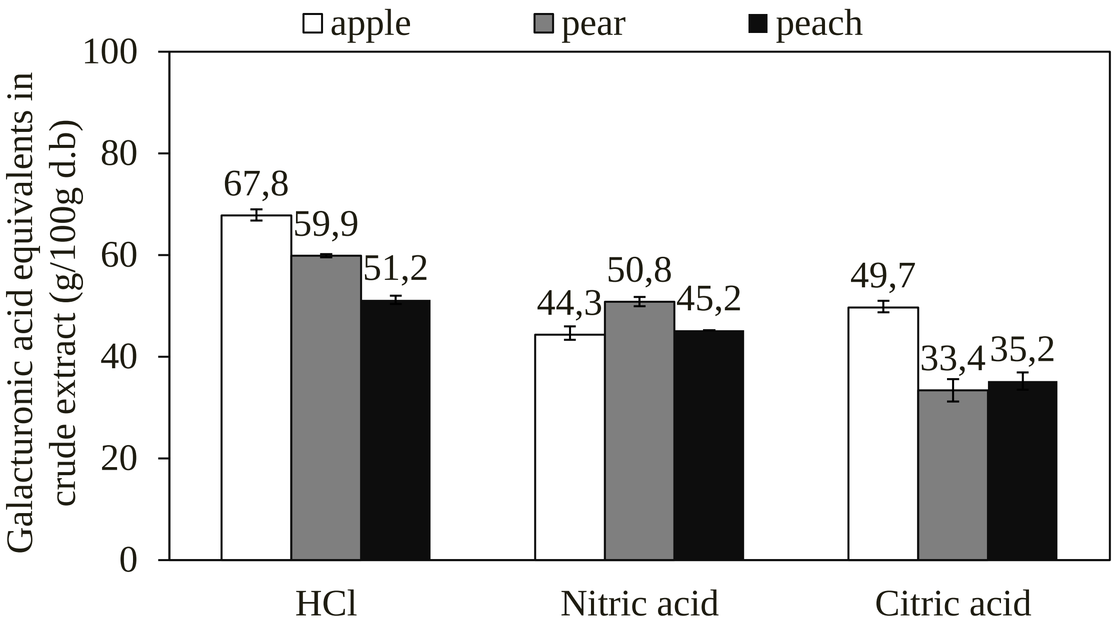
Conditions	
Solvent	70% ethanol + 0.5% HCL
Solid to Solvent ratios (w/v)	1:10,1:20,1:30
Duration	30 min × 3 times



- High total phenolic content in apple (~902 mg GAE/ 100 g dw)
- Better total phenolic content extraction in solid to solvent ratio 1:30

Extraction of pectins



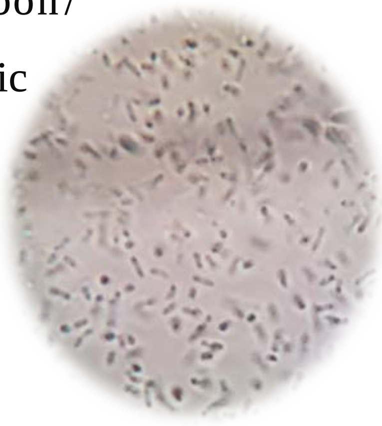


Conditions	
HCl	pH:2 ,75^{0}C, 60 min, solid to solvent ratio 1:25 (w/v)
Citric acid	pH 2, 87^{0}C, 160 min, solid to solvent ratio 1:25 (w/v)
Nitric acid	pH:2 ,80^{0}C, 70 min, solid to solvent 1:25 (w/v)

- Highest pectin yield was obtained in apple and pear substrates using HCl

Evaluation of nitrogen source in shake flasks fermentations

- Carbon source : Glucose, Fructose, Sucrose, Free sugars
- Microorganism : *Burkolderia sacchari* DMSZ 17165
- Fermentations were carried out at four carbon/ nitrogen ratios (C/N:160, 80, 40 ,20) in organic nitrogen source (yeast extract) and two carbon/ nitrogen ratios (C/N: 20:1, 10:1) in inorganic nitrogen source ($(NH_4)_2SO_4$)



Batch fermentations in shake flasks

	Organic Nitrogen Source	Inorganic Nitrogen Source
Carbon source (g/L)	40	40
Yeast extract (g/L)	1, 2.5, 6.15, 15.0	1
$(NH_4)_2SO_4$ (g/L)	1	2-4
$Na_2HPO_4 \cdot 2H_2O$ (g/L)	4.5	4.5
KH_2PO_4 (g/L)	1.5	1.5
$MgSO_4 \cdot 7H_2O$ (g/L)	0.2	0.2
Trace element (mL/L)	1	1

Trace element: solution (per L): $FeS0_4 . 7H_20$, 10 g; $ZnS0_4 . 7H_20$, 2.25 g; $CuSO_4 . 5H_20$, 1 g; $MnS0_4 . 4\text{-}5H_20$, 0.5 g; $CaC1_2 . 2H_20$, 2 g; $Na_2B_40_7 .10H_20$, 0.23 g; $(NH_4)_6Mo_70_{24,}$ 0.1 g; 35% HC1 10 mL.

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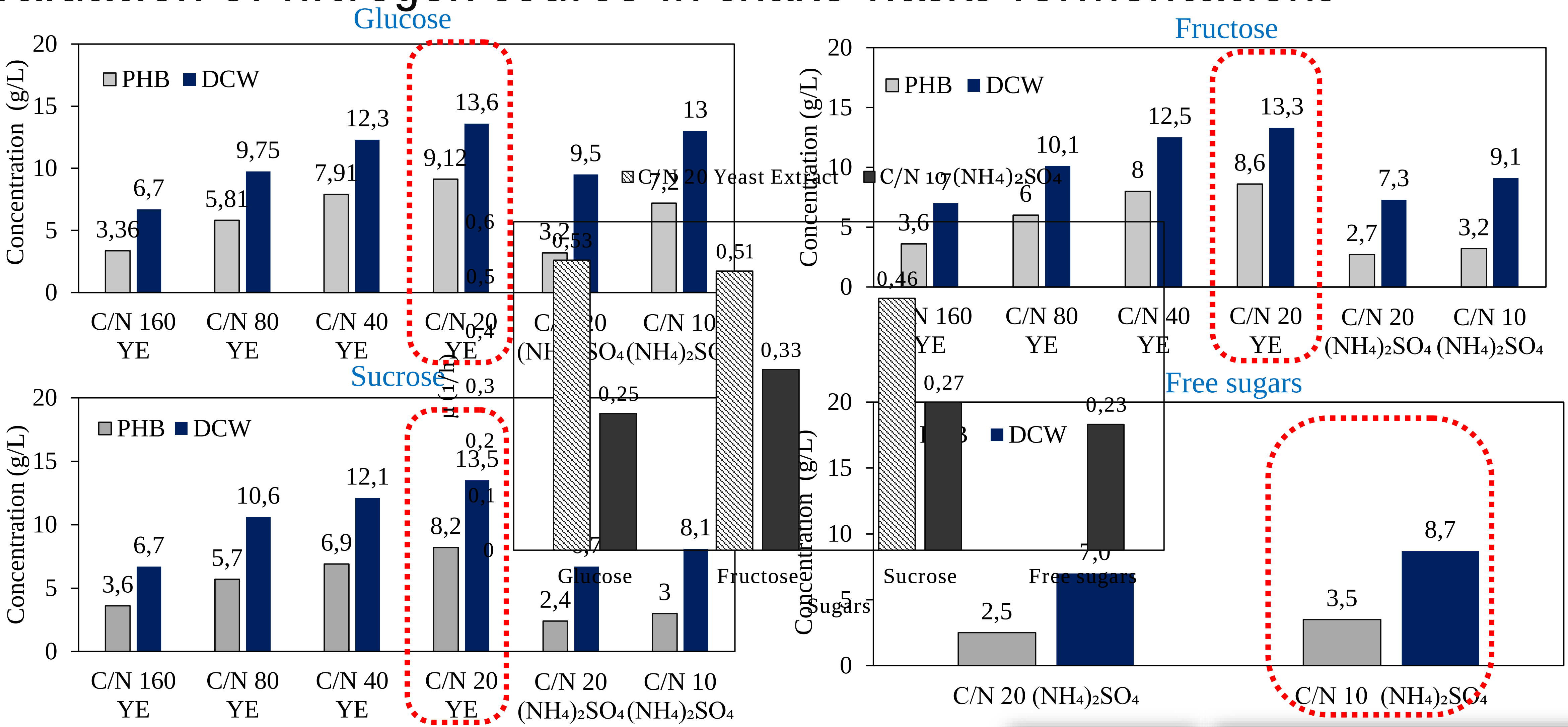
Evaluation of nitrogen source in shake flasks fermentations



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Fed batch fermentation with phosphorous limitation

Fed Batch Fermentation	
Carbon Source	Free Sugars 40 g/L
Working volume	1 L
pH	6.8 (28% NH_4OH και 2M HCl)
Temperature	30
Agitation	Cascade 400-1200 rpm, DO: 20 %
Aeration	2.5 vvm
Composition (g/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. **Trace element (g/L):** $FeSO_4 \cdot 7H_2O$, 10 g; $ZnSO_4 \cdot 7H_2O$, 2.25 g; $CuSO_4 \cdot 5H_2O$, 1 g; $MnSO_4 \cdot 4H_2O$, 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% HCl 10 mL

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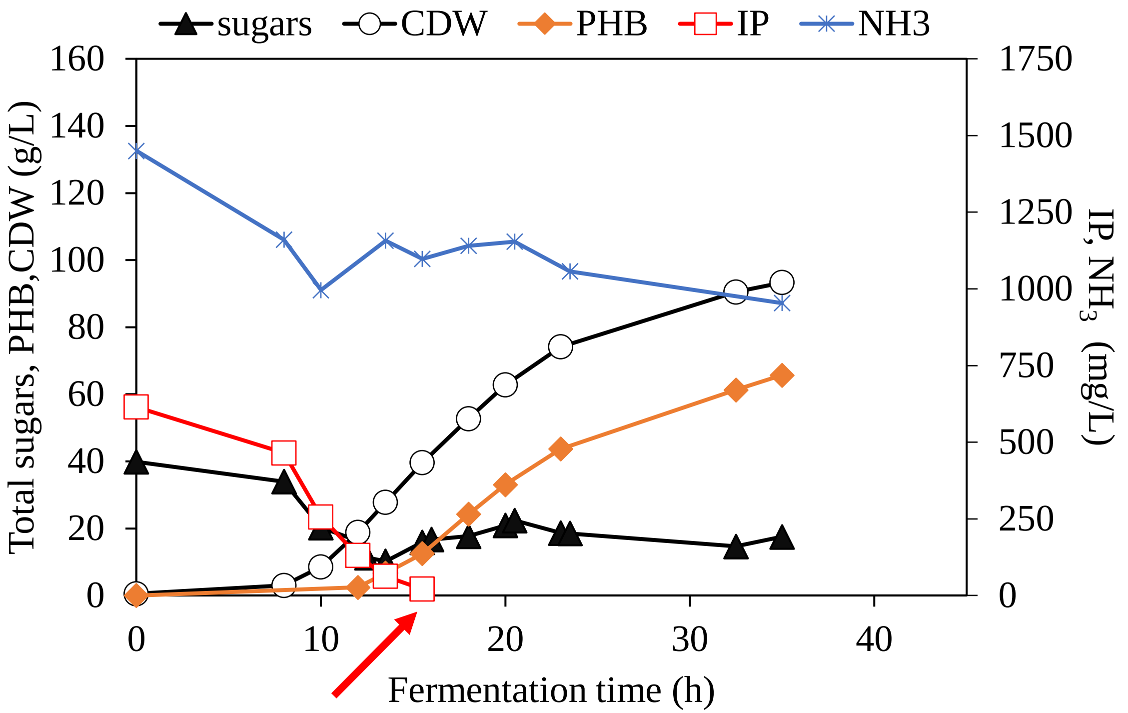
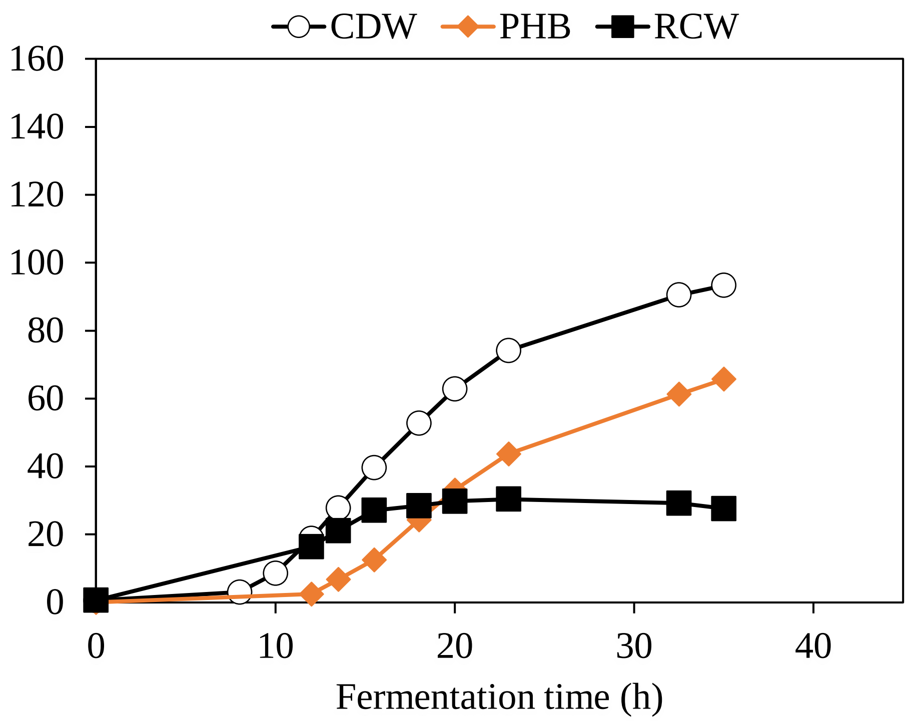
Fed batch fermentation with phosphorous limitation strategy





Time (h)	CDW (g/L)	RCDW (g/L)	P(3HB) (%)	P(3HB) (g/L)	Yield (g PHB/g consumed sugars)	Yield (g biomass/g consumed sugars)	Productivity (g/L/h)
35	93.3	27.6	70.4	65.7	0.34	0.15	1.88

Conclusions

- The recovery of value-added components from Fruits wastes could increase the profitability of fruit wastes-based biorefinery development
- Batch fermentations: Ammonium sulphate as inorganic nitrogen source can be utilized as nitrogen source
- Fed batch fermentation: Free sugars from fruit wastes was efficiently valorized as carbon source for P(3HB) production

Thank you for your attention!

“Bioconversion of Food Industry Wastes to Biopolymers for Packaging Applications in a Biorefinery Concept - Wastes-to-Biopolymers”

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