2nd Conference on Green Chemistry and Sustainable Coatings

greenchemco

*Pisa, Italy, 28th-30th June 2023*

# From food waste to fine chemicals: hydrolysis of defatted wheat bran and sugars conversion to lipids and carotenoids by fermentation

Nicola Di Fidio[1], Getari Kasmiarti[2], Leonardo Carmassi[1], Domenico Licursi[1], Sara Fulignati[1], Claudia Antonetti[1], Anna Maria Raspolli Galletti[1]

[1]*Department of Chemistry and Industrial Chemistry, University of Pisa, Via G. Moruzzi 13, Pisa, Italy*
[2] *Doctoral Program of Environmental Science, Graduate Program Universitas Sriwijaya, Jalan Padang Selasa No.524, Bukit Besar, Palembang 30139, South Sumatra, Indonesia*
*Telephone: +39 0502219210, nicola.difidio@unipi.it*

Keywords (Max 5): wheat bran, biorefinery, sugars, lipids, carotenoids

**Introduction**

The transition from a linear economy based on the exploitation of fossil resources to a bio-based circular economy is an urgent global goal. The exploitation of waste biomasses into added-value bio-chemicals within innovative biorefinery schemes is strongly encouraged. Among biomasses defatted wheat bran represents a strategic food-chain waste since it is a by-product of milling and is characterized by a high content of polysaccharides and very low content of lignin. Moreover, it cannot be used for animal feed due to its low content of proteins. For these reasons, an innovative cascade biorefinery was designed and optimized to produce fermentable sugars by enzymatic hydrolysis and high added-value fine chemicals, namely carotenoids and lipids, by fermentation, adopting the safe commercial yeast *Rhodosporidium toruloides*. Carotenoids present commercial applications in food, pharmaceutical, nutraceutical and cosmetic industries as well as in the field of innovative materials for electronics such as transistor. Similarly, lipids are an important industrial platform chemical to produce biofuels (e.g., biodiesel), animal feed, bioplastics, biosurfactants, additives and lubricants.

**Experimental**

Defatted wheat bran was provided by Casillo Next Gen Food company (Puglia, Italy). The complete chemical composition of raw feedstock was evaluated through the standard NREL protocols. For the enzymatic hydrolysis of defatted wheat bran, the commercial enzymatic mixture Cellic CTec 3 HS was employed as catalyst. The effect of different biomass loadings (2, 10, 15 and 20 wt%) and enzyme dosages (15, 30, 45, 60 FPU/g glucan) on the sugars yield was investigated. For the fermentation step, the commercial safe yeast strain *Rhodosporidium toruloides* DSM 4444 was adopted as biocatalyst. The effect of the C/N ratio and the carbon source on carotenoids and lipids production was investigated on synthetic model media and real biomass hydrolysates. Carotenoids were extracted by standard protocol based on the use of DMSO for cell disruption and hexane for carotenoids selective extraction in the absence of light. Carotenoids content was measured spectrophotometrically using β-carotene as standard. After carotenoids extraction, lipids purification was performed by the standard method and the total amount of lipids was determined gravimetrically.

**Results and Discussion**

Figure 1 shows the main results of the enzymatic hydrolysis of wheat bran.

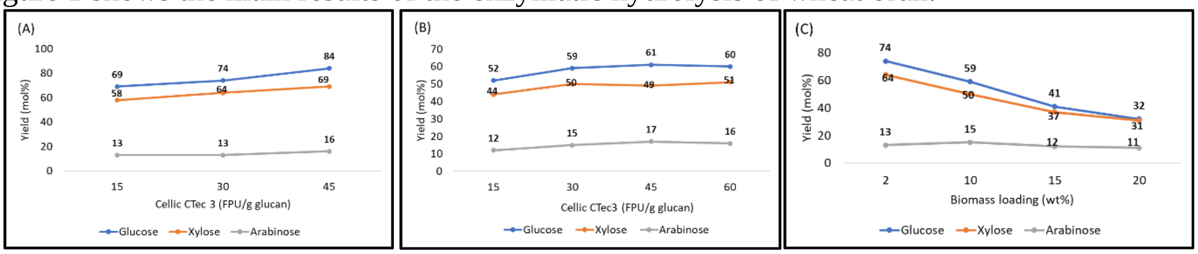


**Figure 1.** Effect of the enzyme dosage on sugars yield with a biomass loading of 2 wt% (A) and 10 wt% (B); Effect of biomass loading on sugars yield using 30 FPU/g glucan Cellic CTec 3.

Figure 2 shows the fermentation profile (sugars consumption and cell growth) of wheat bran hydrolysate.

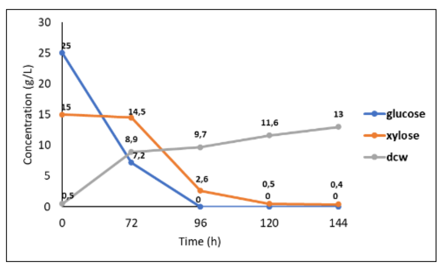


**Figure 2.** Consumption profile of sugars and dry cell weight profile during the fermentation.

**Conclusions**

In the perspective of the circular bioeconomy, for the first time, an integrated biorefinery model was developed to convert the defatted wheat bran to sugars (glucose, xylose, arabinose), triacylglycerols, carotenoids, obtaining a protein-rich solid residue potentially suitable for animal feed. Under the optimal conditions of the enzymatic hydrolysis reaction, the yields of glucose, xylose and arabinose were 60, 50 and 15 mol%, respectively, corresponding to ca. 40 g/L as total reducing sugars. *Rhodosporidium toruloides* converted all sugars into carotenoids and triglycerides achieving the production values of ca. 180 mg/L and 1.6 g/L, respectively. The single cell oil profile was similar to that of vegetable oil and the integrated production of these fine chemicals increased the economic sustainability of the process.

**Acknowledgments:**

This research was conducted within the activities of the RTD-A contract (no. 1112/2021, Prot. no. 0165823/2021) co-funded by the University of Pisa in respect of the PON "Ricerca e Innovazione" 2014-2020 (PON R&I FSE-REACT EU), Azione IV.6 "Contratti di ricerca su tematiche Green". Dr. Marcello Miani of Casillo Next Gen Food company is kindly acknowledged for providing the biomass.

**Short Author's biography**

Dr. Nicola Di Fidio is Junior Researcher in Industrial Chemistry at the Department of Chemistry and Industrial Chemistry (DCCI) of the University of Pisa. He holds the course of "Industrial Biotechnologies" within the Master's Degree course in "Chemistry" and in "Industrial Chemistry" at the DCCI.
Dr. Di Fidio received his MSc degree "cum laude" in "Industrial and Environmental Biotechnologies" in 2015 at the University of Bari. In 2016 he worked at the Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA – Trisaia) in Basilicata. In 2017 Dr. Di Fidio was research fellow at the Department of Bioscience, Biotechnology and Biopharmaceutics of the University of Bari. In 2018 he won a fellowship at the Doctoral School in Chemistry & Material Science (University of Pisa) and in 2021 he received the PhD degree "cum laude". In the same year, he performed postdoctoral research at DCCI in Industrial Chemistry. Since 2022 Dr. Di Fidio is junior researcher.
His research activity is focused on chemical and biological catalytic approaches for the sustainable valorisation of agro-industrial wastes and residual biomasses in the perspective of the principles of Green Chemistry and Circular Economy. In particular, Di Fidio's projects regard the design, the implementation and the optimisation of innovative and tailored biorefinery processes, based on the synergistic combination of Chemistry and Biotechnology, to produce bio-fuels (e.g., biodiesel, alkyl levulinates) added-value fine chemicals (lipids, organic acids, furan derivatives, carotenoids, polysaccharides) and materials (e.g. microporous activated carbon).