Microbial oil and carotenoid production by oleaginous yeast using vegetable and urban waste

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Nowadays, the high generation of waste in different sectors and industries is an environmental, management and handling, and even economic problem. The reincorporation of these residues into new work cycles or processes as profitable raw materials would have environmental and economic advantages due to the non-exploitation of new resources and the low cost of waste for its recovery, recycling or reuse. This study aims to use waste as raw material in a biotechnological process in which products with high-added value are obtained; in such a way, a value and an outlet are given to material that is considered waste. In this case, the residues come from intensive vegetable and fruit agriculture and the urban sector. Specifically, work has been carried out with discarded pepper, urban pruning and rejected paper (not recycled). From these raw materials, culture media were generated to grow oleaginous yeasts. Specifically, the oleaginous yeast *Rhodosporidium toruloides* accumulates lipids under certain stress conditions (e.g., low N concentrations) and naturally produces carotenoids, high value-added and marketable products.

For lipids and carotenoid production, bioreactor-scale studies have been carried out. A fed-batch strategy was followed, and the different residues used were compared. Firstly, the inoculum was prepared by growing *R. toruloides* at 180 rpm and 28ºC on YPD media. After 24 h, cells were harvested by centrifugation at 5000 *g* for 5 min, washed and diluted with distilled water to obtain the desired inoculum concentration. Then, a 0.5 L bioreactor (Minibio, Applikon) was used, with 0.25 L of the soluble fraction of pepper obtained after the crushing and centrifuging strategy. The pepper-derived medium was used as a growth medium in the fed-batch because it is rich in different carbon sources and other nutrients. The culture temperature was maintained at 30ºC and pH-controlled at 6, and aeration was maintained by adjusting agitation speed with an airflow rate of 1vvm (> 20 % of air saturation). Prior to the total depletion of the carbon source and to increase the C/N ratio to promote microbial oil accumulation, pulsed carbon source additions were applied. Glucose obtained from enzymatic hydrolysis of the pretreated municipal wastes (pruning or paper) was used as a pulsed carbon source. The concentration of sugars, the dry cell mass, and the lipid composition were monitored periodically. Sugars concentration was determined by HPLC chromatography, and dry cell weight was measured by gravimetric method. Lipid content was measured as total FAME after whole biomass in situ transesterification (Van Wychen et al., 2016). Total lipids and carotenoid content were determined by gravimetric and spectrophotometry, respectively.

Finally, after extractions and quantifications of microbial oils following the fed-batch strategy, more than 50% of total lipids have been achieved in both assays (with pruning and paper). Furthermore, the fatty acids profile obtained, where oleic, palmitic, and linoleic acids predominate (Figure 1), was similar to the profile of vegetable oils used for biodiesel production.

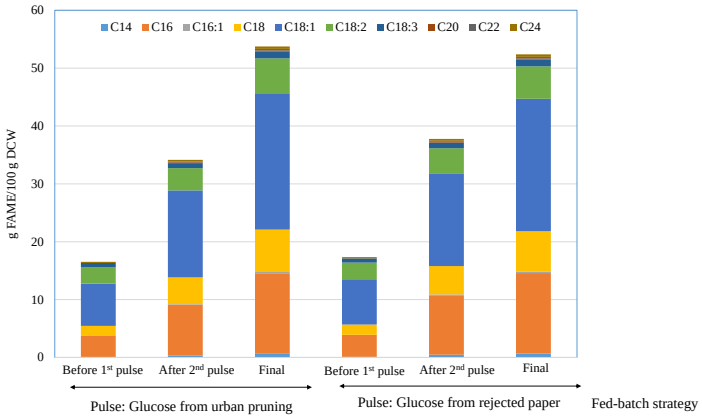

Figure 1. Fatty acid composition of lipids from *R. toruloides* at different culturing stages. Myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), behenic acid (C22:0), lignoceric acid (C24:0).

It is concluded that the medium derived from discarded pepper is suitable for cell biomass production, and both the medium derived from urban pruning and rejected paper leads to similar results and are suitable for intracellular bioproduct accumulation.

References:

Van Wychen, S., Ramirez, K., & Laurens, L. M. Determination of Total Lipids as Fatty Acid Methyl Esters (FAME) by in situ Transesterification: Laboratory Analytical Procedure (LAP). 2016.

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