Lipid production by oleaginous yeast using vegetable waste from sorting process

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Single Cell Oils obtained from oleaginous yeast are expected to be of great interest in biofuel and oleochemical industries in the near future. However, cost-effective microbial oil production requires low-cost substrates.

In southwestern Spain, the horticultural intensive type systems dedicated to producing greenhouse vegetables represent one of the main industries generating organic wastes. It is estimated that the losses of fruit and vegetable handling, storage and transport for Europe are around 5% of the total production. Currently, the main destination for organic waste derived from the horticultural sector is animal feed, especially cattle and sheep. Due to their high moisture content, these residues cannot be stored and therefore have to be utilised quickly. On the other hand, in areas where there is no livestock, organic waste is transferred to external waste management companies and authorised recycling plants for their treatment and recovery process, implying an extra cost to the farms (Duque-Azevedo *et al*., 2020). Hence, residues from the agro-food industries as biomass feedstocks for oleaginous yeast cultivation represent an attractive alternative with potential application in the energy sector.

After analysing the chemical composition of these residues, it was observed that the sugar content varies from 50 to 80% (d.w), which highlights its potential as a source of carbon for different bioprocesses such as microbial oil production using oleaginous yeasts. Oleaginous yeasts are characterised by having the ability to accumulate more than 20% lipids to their cellular biomass (Ma *et al.,* 2018). However, under certain growing conditions such as high carbon/nitrogen (C/N) ratios, these yeasts can reach lipid accumulations of more than 70% over their dry weight (Thevenieau and Nicaud, 2013)

In this work, the vegetable from discarded (pepper, tomato and watermelon) was submitted to a crushing step, followed by a centrifugation step. After centrifugation, the supernatant was analysed for sugars content by HPLC and used as a carbon source for lipid production.

Cryptococcus curvatus (CL6032 from BBN-ISCIII) was used as a microorganism for lipid production. The inoculum was prepared by growing the yeast on an orbital shaker incubator at 180 rpm and 25ºC on YPD media. After 24 h, cells were harvested by centrifugation at 5000 *g* for 5 min, washed once with 0.9% NaCl, and diluted with distilled water to obtain the desired inoculum concentration. For lipid production, a 0.5L bioreactor (Minibio, Applikon) contained 0.25 L of the soluble fraction obtained after crushing and centrifugation of pepper, tomato and watermelon discarded residues. The culture temperature was maintained at 28ºC and pH-controlled at 6, and aeration was maintained by adjusting agitation speed with an airflow rate of 1vvm (> 20% of air saturation). In order to increase C/N ratio, feed-batch strategy cultivation with pulsed carbon source addition were applied. For that, a concentrate glucose solution was added in pulse when the fermentation broth's carbon source was below 5 g/L. The concentration of sugars, the dry cell mass, and the lipid composition were sampled periodically. Sugars were determined by HPLC, dry cell mass was measured by gravimetric method. Lipid content was measure as total FAME after whole biomass in situ transesterification (Van Wychen *et al*., 2016). FAME was analysed by gas chromatography with a flame ionisation detector (GC-FID)

Available sugars in the soluble fraction obtained from discarded pepper, tomato and watermelon were 40.5 g/100 g dry matter, 39.4 g/100 g dry matter and 63.3 g/100 g dry matter, respectively. The sugar concentration in this soluble fraction was 58 g/L (pepper), 25 g/L (tomato) and 65g/L in watermelon residue. A summary of the results obtained of lipid production are shown in table 1.

Table 1. Summary of yields after fed-batch cultivation on different substrates

	DCW (g/L)	Lipid (g/L)	Lipid content (%,w/w)	Lipid yield (g lipid/g sugar consumed	Cell mass yield (g yeast/g sugar consumed)
Discarded pepper	47.3	16.8	32.7	0.106	0.202
Discarded Tomato	23.8	9.2	37.8	0.097	0.249
Discarded watermelon	41.8	13.3	29.5	0.101	0.316

Cell mass production ranged from 23.8 to 47.3 g dry cell weigth/L, reaching the highest value with pepper residues. The highest lipid content (37.9%) was found when soluble sugar from tomato discarded were utilized as substrate, but the lowest lipid yield was obtained.

Lipid profiles obtained from different substrates are depitected in figure 1.

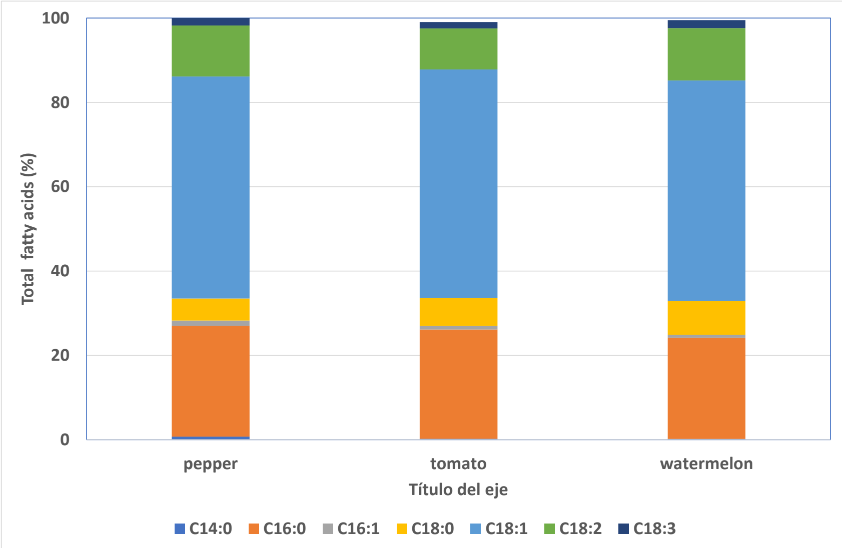

Figure 1. Comparison of fatty acid composition of lipids from *C. curvatus* grown on different substrates. Myristic acid (C14:0), palmitic acid (16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (18:3).

The fatty acid profile achieved in all trials has been very similar in all substrates, predominating oleic acid and palmitic acid; accounting for about 80% of the total fatty acids produced.

Sugars content in vegetables can be easily extracted by mechanical methods (crushing and centrifugation). Watermelon was found to have the highest content of sugars in the soluble fraction. Lipid concentrations up to 16.8 g/L were obtained from the soluble fraction from discarded pepper and pulsed glucose addition in a feed-batch strategy cultivation. Furthermore, the fatty acids profile obtained was similar to the profile of vegetable oils used for conventional biodiesel production.

References:

Duque-Azevedo, M.; Belmonte-Ureña, L.J.; Plaza-Úbeda, J.A. The management of agricultural waste biomass in the framework of circular economy and bioeconomy: an opportunity for green house agriculture in Southeast Spain. Agronomy 2020, 10, 489.

Ma, Y., Gao, Z., Wang, Q., Liu, Y. Biodiesels from microbial oils: Opportunity and challenges. Bioresour. Technol. 2018, 263, 631–641.

Thevenieau, F., & Nicaud, J. M. (2013). Microorganisms as sources of oils. OCL - Oilseeds & fats, Crops and Lipids, 2013, 20(6), D603.

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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