# Simultaneous production of carotenoids and microbial lipids using fruit wastes from open markets

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## Introduction

Studies on consumers' behaviour have shown that food coloration is a critical choice parameter. At the same time, consumers demand natural products, with high organoleptic characteristics and nutritional value. According to a recent industrial report, the estimated global food colors market for 2016 accounted USD 1.79 billion, with a growth of 5.9% (Anonymous, 2020). Considering carotenoids, the world market in the year 2014 amounted $1.4 billion for approximately 1,400 metric tons (wwww.deinove.com). Microbial lipids have attracted the scientific interest mainly as alternative feedstock for biodiesel production, since their fatty acid composition is similar to vegetable oils (Guo et al., 2016). Besides fuel production, microbial lipids can also be utilized for the production of value-added chemicals with various applications, such as novel oleogels (Ochsenreither et al., 2016; Papadaki et al., 2019). For the sustainable production of both carotenoids and microbial oils the use of renewable resources would serve as an economically efficient alternative. In this respect, fruit wastes could serve as an inexpensive substrate for microbial fermentations

The yeasts belonging to *Rhodotorula* sp. and *Rhodosporidium* sp. are well known producers of both carotenoids and microbial oils (Papadaki et al., 2019). However, the studies evaluating their simultaneous accumulation by yeast strains are scarce. In this study, three yeast strains (*Rhodotorula glutinis*, *Rhodosporidium toruloides* and *Rhodosporidium kratochvilovae*) were evaluated for their ability to produce both carotenoids and lipids when cultivated in fruit aqueous extracts. Fruits (mainly peaches and nectarines) discarded from open markets were initially fully characterised regarding their content in free sugars, phenolic compounds, pectins, cellulose, hemicellulose and lignin. Free sugar extraction was optimized and the extracts were subsequently utilised as fermentation feedstocks for carotenoids and microbial lipids production. To the best of our knowledge, this is one of the first studies that fruit residues are investigated as potential inexpensive substrate for simultaneous or sequential production of carotenoids and microbial lipids.

## Materials & Methods

Fruits (peaches and nectarines) were collected from a local open market in Athens, Greece. The collected fruits were first macerated and then homogenized using a domestic blender. Free sugars were extracted using deionized water at a solid-to-liquid ratio 1:10 at three temperatures (30 $^oC$, 40 $^oC$ and 45 $^oC$). Extractions were carried out 3 times and the derived aqueous extracts were utilised as fermentation substrates. Cellulose, hemicellulose and lignin contents were determined following the protocol of Sluiter et al. (2011). Extraction of phenolic compounds and pectins were carried out according to the protocol described in the study of Tsouko et al. (2019) with 1:20 solid-to-solvent ratio and Melton and Smith (2001), respectively.

The oleaginous yeasts *Rhodotorula glutinis* NRRL 252, *Rhodosporidium toruloides* DSMZ 4444 and *Rhodosporidium kratochvilovae* FMCC Y-43 were utilised for the simultaneous production of carotenoids and microbial lipids. *R. glutinis* was purchased from the ARS Culture Collection (NRRL) and *R. toruloides* was obtained from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures. The strain *R. kratochvilovae* has been isolated from fish and belongs to the Food Microbiology Culture Collection (FMCC) in the Laboratory of Food Microbiology and Biotechnology in the Agricultural University of Athens (Greece). The yeasts were maintained in Petri dishes with YPD medium (yeast extract, peptone, and dextrose) and 2% agar. Pre-cultures were prepared in YPD medium and incubated at 28 $^oC$ in an orbital shaker at 180 rpm for 24 h.

Shake flask fermentations, using free sugars from fruits as carbon source, were carried out in 250 mL Erlenmeyer flasks with 50 mL medium. Inoculum was 10% (v/v) in all cases. Fermentations were performed in an orbital shaker at 28 $^oC$ and an agitation set to 180 rpm. The pH was maintained in the range of 5.5-6.2 using 5M NaOH. In all the experiments, initial total sugar concentration was around 70 g/L and the fruit aqueous extracts were also supplemented with yeast extract (0.5 g/L) and $(NH_4)_2SO_4$ (0.5 g/L) as nitrogen sources and with phosphate salts (7 g/L $KH_2PO_4$, 2.5 g/L $Na_2HPO_4$). The effect of the addition of trace elements (in g/L: $MgSO_4{\cdot}7H_2O$, 1.5; $CaCl_2{\cdot}2H_2O$, 0.15; $FeCl_3{\cdot}6H_2O$, 0.15; $MnSO_4{\cdot}H_2O$, 0.06; $ZnSO_4{\cdot}7H_2O$, 0.02) was also investigated.

Samples were taken at regular intervals in order to monitor sugar, nitrogen and inorganic phosphorus consumption, cell growth, carotenoids and microbial lipids production. All methods are presented in detail in the study of Papadaki et al. (2019), except for total carotenoids determination which was carried out according to the protocol developed by Freitas et al. (2014).

**Results & Discussion**

Free sugar extraction at 40 ºC resulted to slightly higher sugar yields than 30 ºC and 45 ºC. The aqueous extracts contained almost 85.5 g/L total sugars of which 55.1% were sucrose, 25.2% glucose, and 19.7% fructose. These extracts were utilized as carbon source for the cultivation of oleaginous yeast strains in shake flask cultures with or without supplementation of trace element solution. The selected strains grew on the aqueous fruit extract and were able to simultaneously accumulate lipids and carotenoids. All sugars were completely consumed in about 145 h with dry cell weights (DCW) being in the range of 23-30 g/L. Sucrose was rapidly hydrolysed into glucose and fructose in all studied strains, with glucose being consumed before fructose. Interestingly, the production of DCW, total carotenoids and lipids was similar for all strains, regardless the addition or not of trace elements. The highest lipid and carotenoid production was achieved with the strain *R. toruloides*, presenting total carotenoids content of more than 200 μg/g DCW and more than 50% lipid content. The yeast cells started accumulating carotenoids, almost at the same time as lipids, at ca. 48 h. The onset of lipid and carotenoid accumulation coincided with the complete consumption of free amino nitrogen. In conclusion, aqueous fruit extracts could be utilized as an inexpensive substrate for the production of carotenoids and microbial lipids. Among the three studied yeast strains, *R. toruloides* presented the highest potential for both lipids and carotenoids production.

**References**


Anonymous, 2020. www.grandviewresearch.com/industry-analysis/food-colorants-market

Freitas C., Nobre B., Gouveia L., Roseiro J., Reis A., Lopes da Silva T. 2014. New at-line flow cytometric protocols for determining carotenoid content and cell viability during *Rhodosporidium toruloides* NCYC 921 batch growth. Process Biochemistry, 49: 554-562.

Guo M., Cheng S., Chen G., Chen J. 2019. Improvement of lipid production in oleaginous yeast *Rhodosporidium toruloides* by ultraviolet mutagenesis. Engineering in Life Sciences, 19: 548-556.

Melton L.D., Smith B.G. 2001. Determination of the uronic acid content of plant cell walls using a colorimetric assay. R.E. Wrolstad, T.E. Acree, E.A. Decker, M.H. Penner, D.S. Reid, S.J. Scwartz, *et al.* (Eds.), Current protocols in food analytical chemistry (Unit E3.3), John Wiley & Sons Inc, Hoboken, NJ, USA

Ochsenreither K., Glück C., Stressler T., Fischer L., Syldatk C. 2016. Production strategies and applications of microbial single cell oils. Frontiers in Microbiology, 7: 1-26.

Papadaki A., Kopsahelis N., Mallouchos A., Mandala I., Koutinas A.A. 2019. Bioprocess development for the production of novel oleogels from soybean and microbial oils. Food Research International, 126: 108684.

Sluiter A., Hames B., Ruiz R., Scarlata C., Sluiter J., Templeton D., Crocker D. 2011. Determination of structural carbohydrates and lignin in biomass. Technical Report. NREL/TP-510-42618,

Tsouko E., Alexandri M., Fermandes K.V., Guimarães Freire D.M., Mallouchos A., Koutinas A.A. 2018. Extraction of phenolic compounds from palm oil processing residues and their application as antioxidants. Food Technology and Biotechnology 57 (1): 29-38.


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