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RESEARCH

Biotechnological conversion of olive mill wastewater and cheese whey into functional compounds with *Rhodotorula glutinis*

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Abstract

Olive mill wastewater (OMWW) and cheese whey (CW) are two agro-industrial effluents that pose major environmental challenges due to their high organic load and potential environmental impact. This study investigates a novel valorization approach by cultivating the oleaginous yeast *Rhodotorula glutinis* on OMWW supplemented with CW, a nitrogen-rich dairy byproduct. Four OMWW/CW ratios (25/75, 50/50, 75/25, 100/0, v/v) were evaluated to assess their impact on microbial growth, metabolite production, and wastewater remediation. The 25/75 (v/v) and 50/50 (v/v) mixtures supported the highest biomass yields (>5 g L^{-1} within 3—5 days), while the 50/50 (v/v) mixture led to maximum lipid and carotenoid accumulation after 8 days. Under this condition, substantial depollution was also observed, with near-complete removal of hydroxytyrosol and tyrosol as well as significant reduction in chemical oxygen demand (COD). Carotenoids extracted from *R. glutinis* showed thermal stability at 60 °C and exhibited strong antioxidant and antibacterial activities. These findings demonstrate the feasibility of coupling waste treatment with the production of bioactive compounds. The proposed bioprocess valorizes two problematic waste streams into valuable microbial biomass and functional metabolites, with potential to reduce environmental impact.

Highlights

- OMWW and CW were co-utilized as an innovative fermentation medium for *Rhodotorula glutinis* cultivation.
- A 50/50 OMWW/CW (v/v) mixture maximized lipid and carotenoids production after 8 days.
- Significant COD and phenolic compound removal was achieved in the 50/50 OMWW/CW (v/v) mixture.
- Crude carotenoids extract showed thermal stability and strong antioxidant activity.

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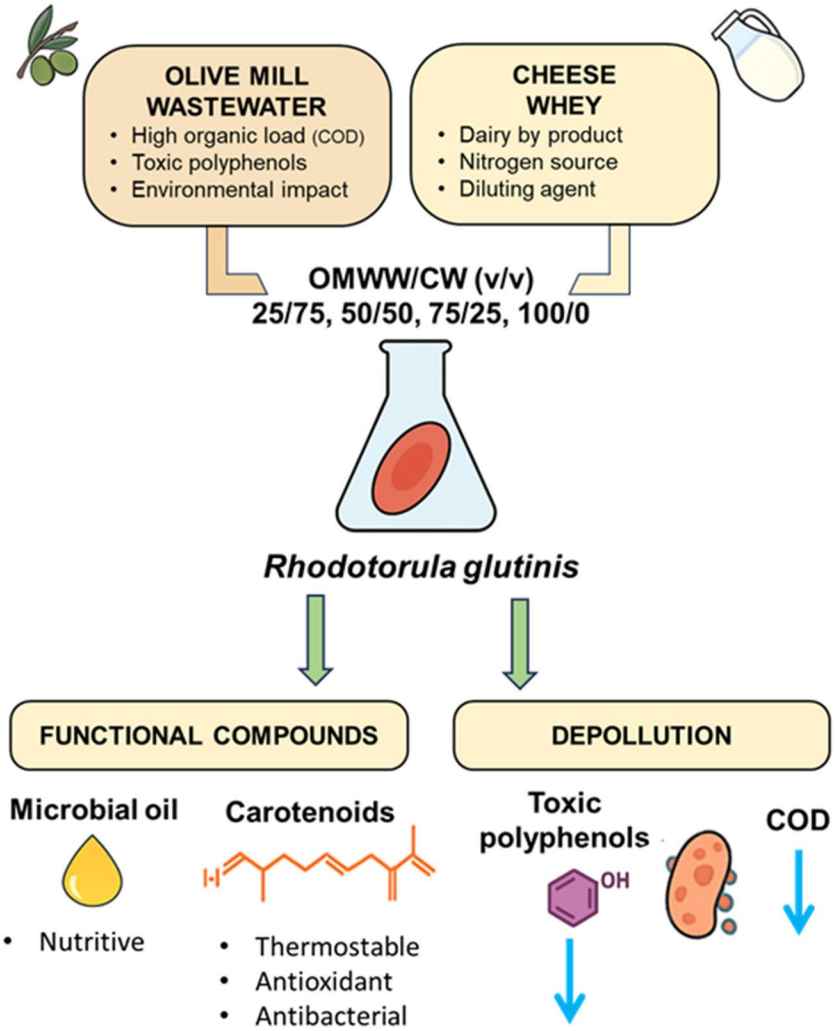
Graphical abstract



Keywords Olive mill wastewater · Cheese whey · *Rhodotorula glutinis* · Carotenoids · Lipids

Introduction

The Mediterranean region is widely recognized as the main hub for olive oil production worldwide. In 2019, 3.314 million tons of olive oil were produced globally, 98% of which derived from the Mediterranean region (Kaniewski et al. 2025). Algeria produced over 50,000 tons of olive oil in 2022, highlighting its role in this vital sector (Chikhi and Bendi Djelloul, 2022).

However, the production of olive oil results in significant quantities of a heavily polluted liquid waste known as olive mill wastewater (OMWW), primarily due to the high volume of water used during extraction (Al-Bsoul et al. 2020). The amount of OMWW generated typically ranges from 0.3 to 1.2 m^3 per ton of olives processed, depending on the extraction technique employed. The three-phase centrifugation technique is the most water-intensive, as it requires additional water to improve the fluidity of the olive paste. For example, processing one ton (1000 kg) of olives using this method typically requires about 1000 kg of added water and produces approximately 200 kg of olive oil, 600 kg of solid pomace, and 1200 kg of wastewater (Morillo et al. 2009). Global OMWW generation is estimated to range between 10 and 30 million m^3 annually (Akkam et al. 2024). In Algeria, thousands of cubic meters of OMWW are produced alongside significant quantities of solid waste during olive oil processing (Bougherara et al. 2021).

OMWW presents a major environmental concern due to its low biodegradability, elevated organic loading, and high levels of phenolic compounds, which are known to be toxic to plants and inhibitory to soil microbial communities (Fleyfel et al. 2024; Fernandes et al. 2025).

Traditional OMWW management generally involves no treatment, crude spreading on agricultural land, or evaporation ponds, all of which are associated with uncontrolled leachate release, soil and groundwater pollution, agricultural productivity reduction, greenhouse gas emissions, and odor problems (Khdair et al. 2017; Ammari et al. 2025).

Over the years, several treatment strategies have been proposed to mitigate the negative impact of OMWW, including processes such as electrocoagulation (Shahawy et al. 2021) and the electro-Fenton technique (El-Gohary et al. 2009). Other advanced technologies assessed for their potential in OMWW treatment include reverse osmosis (Petrotos et al. 2016), electrochemical oxidation (Martinez-Cruz et al. 2020), catalytic peroxidation (Esteves et al. 2022), distillation processes (Jaradat et al. 2018), and thermal conversion (Crialesi et al. 2022).

In parallel, biotechnological approaches are increasingly considered as complementary rather than competing alternatives to physico-chemical methods, since microbial systems not only aid in pollutant removal but also enable resource recovery through the production of value-added compounds (Chiavola et al. 2014). In particular, these processes do not require chemical additives and operate under low-energy conditions, thereby reducing both chemical demand and overall environmental burden (Mohiuddin et al. 2024). Moreover, their CO_2 emissions are expected to be substantially lower than those of evaporation or crude spreading, since the organic load of OMWW is biologically valorized rather than discharged untreated (Enaime et al. 2024).

Recently, the use of OMWW as a substrate for microbial cultivation has emerged as a promising alternative offering both waste treatment and valorization opportunities (Foti et al. 2021; Paz et al. 2023). When appropriately selected, microorganisms such as bacteria (Aouidi et al. 2009; Zavra et al. 2024), yeasts (Arous et al. 2016; Ghilardi et al. 2020; Keskin et al. 2023), and microalgae (Markou et al. 2012; Lenzuni et al. 2025) can grow on OMWW-based media, simultaneously producing biomass and contributing to the biodegradation of toxic compounds like hydroxytyrosol and tyrosol.

Nonetheless, previous studies have consistently reported that direct cultivation in raw OMWW is hindered by the high concentration of polyphenols, which exert toxic and inhibitory effects on microbial growth. To overcome this limitation, acclimatization strategies have been explored; for example, Arous et al. (2018) demonstrated that stepwise exposure to increasing OMWW concentration (COD levels of 25, 50, and 75 g/L) enhanced microbial tolerance to the high organic and phenolic content of OMWW. More commonly, OMWW is diluted with water, with optimal microbial activity typically observed at dilutions of OMWW-water (1/4 v:v) (Karakaya et al. 2012; Boutafda et al. 2019; Ghilardi et al. 2020; Ghilardi et al. 2022). Although this procedure facilitates microbial growth, it is water-intensive, particularly in regions facing increasing water scarcity. Moreover, the widespread adoption of such practices conflicts with environmental policies advocating for water conservation (Javan et al. 2024; Garrido-Baserba et al. 2024).

Additionally, OMWW is characterized by a low nitrogen content, necessitating external nitrogen supplementation to balance the carbon-to-nitrogen (C/N) ratio and support microbial proliferation (El Yamani et al. 2019; Xiao et al. 2022). While supplements such as peptone, tryptone, or yeast extract have proven effective in enhancing microbial growth and metabolite production, their high cost limits the feasibility of using OMWW as a microbial substrate (Christwardana et al. 2025). Alternative inorganic nitrogen sources such as urea and ammonium sulfate have shown promise, but they remain non-negligible in terms of expenses, especially at large production scales. Furthermore, these strategies do not eliminate the need for OMWW dilution, and thus still contribute to water consumption (Konzonck et al. 2022). Identifying alternative nitrogen sources that also reduce the water requirements for OMWW dilution is therefore of growing interest. In this context, cheese whey (CW) emerges as a suitable option. Generated in substantial quantities as a byproduct of cheese manufacturing, CW is rich in organic matter, including nitrogen (7–10 g L^{-1}), as well as essential minerals and vitamins, making it appropriate for microbial cultivation. Its valorization as a complementary substrate to OMWW may help reduce water use and address waste management challenges, while aligning with recent advances in food waste valorization for bioactive compounds and product management that reflect the growing interest in transforming diverse industrial side-streams into valuable resources (Ahmad et al. 2019; OECD/FAO 2022; Nazos et al. 2023; Ozcelik et al. 2024; Kamalesh et al. 2025a; Kamalesh et al. 2025b).

The economic potential of these strategies could be notable, as the recovery of high-value products such as carotenoids and lipids could help offset process costs, especially when using agro-industrial wastes as substrates, as reported in previous studies (Elkacmi and Bennajah 2018; Elkacmi and Bennajah 2019; Girometti et al. 2025).

Rhodotorula glutinis is an oleaginous, red-pigmented yeast widely recognized for its robust metabolic versatility and ability to synthesize a broad spectrum of valuable metabolites from a variety of agro-industrial waste substrates. These include industrially relevant enzymes such as phenylalanine ammonia lyase (PAL), exopolysaccharides (EPSs) with biotechnological applications, as well as organic acids and proteins that enhance its nutritional and functional value (Cho et al. 2001; Kot et al. 2016, Zhao and Li 2022). In addition, *R. glutinis* is capable of accumulating high lipid content (up to 70% of its dry weight) under nitrogen-limited

conditions, making it a promising candidate for the production of essential fatty acids such as linoleic and oleic acids. In addition, *R. glutinis* produces valuable carotenoids, including β-carotene, torulene, and torularhodin, with antioxidant, antimicrobial, and potential anticancer properties. Its ability to utilize a broad range of carbon sources, including agro-industrial waste streams, underlines its relevance for microbial bioprocess development. These characteristics make *R. glutinis* a suitable microbial platform for integrated waste valorization targeting both depollution and bioproduct generation (Kot et al. 2018; Kot et al. 2020).

In contrast to previous studies in which *Rhodotorula* species have been cultivated on single industrial effluents or and solid waste such as lignocellulosic residues (Mast et al. 2014; Liu et al. 2025; Machado et al. 2022), starchy wastes (Kot et al. 2017; Sharma and Ghoshal, 2020), Jerusalem artichoke hydrolysates (Zhao et al. 2010, 2011; Wang et al. 2014, 2019), and oily by-products (Saenge et al. 2011; Hong-Wei et al. 2019; Yen et al. 2019; Sineli et al. 2022),

the present study investigates for the first time the co-fermentation of OMWW and CW. While OMWW alone requires organic and inorganic nitrogen supplementation and significant water dilution (Karakaya et al. 2012; Ghilardi et al. 2020; El Yamani et al. 2019, Taskin et al. 2016), the addition of CW provides both nitrogen and complementary organic matter, thereby reducing the need for external supplements and excessive water use. This dual-substrate strategy directly addresses the limitations reported in earlier works, namely substrate toxicity, nutrient imbalance, and water-intensive pretreatment, while valorizing two major agro-industrial effluents simultaneously.

Therefore, the primary objective of this study is to assess the effect of utilizing different OMWW/CW ratios, which result in varying C/N balances, as cultivation media for the oleaginous yeast *R. glutinis*. This study focuses on evaluating biomass production and its composition, specifically the protein, fatty acid, and carotenoid contents. Additionally, the study examines the thermostability and biological activities (antioxidant and antibacterial properties) of the produced crude carotenoids. Furthermore, the depollution efficiency of OMWW under these conditions is also assessed. To the best of our knowledge, this is the first study to explore the combined use of OMWW and CW as a co-substrate system for *R. glutinis* cultivation.

Material and methods

Sampling of substrates

Two distinct types of agro-industrial wastewaters were evaluated as growth substrates in this study. Fresh samples of OMWW were collected from traditional three phases olive mills factories located in Bejaia (Algeria). OMWW samples were collected immediately after disposal to avoid any change in polyphenols (PC) composition and maintained at −20°C to prevent spontaneous fermentation. Prior to utilization, the OMWW underwent centrifugation at 4000 rpm for 30 min at 4 °C to remove suspended materials, then the resulting supernatant was used as growth media formulation. Cheese whey (CW) was sourced from a dairy facility in Blida province (Algeria), that processes recombinant milk. The collected CW was sterilized, after which thermosensitive proteins were removed by centrifugation at 4000 rpm for 30 min. This process resulted in a supernatant, which was subsequently used for media preparation.

Microorganism and preculture

The oleaginous strain *R. glutinis* DSM 70398, kindly provided by DÜZEN Laboratories Company in collaboration with Ankara University - Chemical Engineering Departement (Turkey), was employed in the present study. This strain was maintained at 4 °C on Dextrose-Sabouraud (DS) medium comprising yeast extract (5 g L^{-1}), peptone (5 g L^{-1}), glucose (10 g L^{-1}), and agar (20 g L^{-1}) and supplemented with 10% (v/v) of OMWW. The preculture of *R. glutinis* DSM 70398 was carried out in sterilized DS broth medium supplemented with 10% (v/v) of crude OMWW (6.33 g L^{-1} polyphenols) and incubated in a rotary thermostatic shaker under 150 rpm at 30 °C, as suggested by Karakaya et al. (2012).

Culture conditions

To assess the suitability of the OMWW-CW mixture as a substrate for *R. glutinis*, a batch mode was selected, given its simplicity and suitability for initial substrate screening. Cultivation experiments were conducted in 250- mL Erlenmeyer flasks, each containing 50 mL of different mixtures based on OMWW and CW (Table 1). The initial pH of the cultivation media was adjusted to 5.7 using 12 M NaOH. After sterilization, the flasks were cooled to room temperature and then inoculated with 10% (v/v) of a 48h-aged preculture on DS broth containing 4×10^8 cells mL^{-1} (~0.04 g

Table 1 Characterization of OMWW and CW mixtures

Cultivation Medium code	Mixture (%, v/v)	C/N	PC (g/L) *
OC25	25% OMWW + 75% CW	20:1	1.8 ± 0.12
OC50	50% OMWW + 50% CW	35:1	2.6 ± 0.14
OC75	75% OMWW + 25% CW	72:1	3.3 ± 0.27
OC100	100% OMWW + 0% CW	287.5:1	6.33 ± 0.53

*Determined using folin ciocalteu method (760 nm)

L^{-1}). The cultures were then incubated in a rotary shaker at 150 rpm at 30°C. A sacrificial culturing methodology was employed, with samples collected every 48 h over a 10-day cultivation period. Cultivation was carried out in triplicate for each cultivation medium.

The optimal conditions were further validated at a 5-L scale for large-scale carotenoid production, intended for subsequent characterization, including antioxidant and antibacterial activities, FTIR analysis, thermostability, and UV–Visible spectrophotometry.

Analytical methods

Analyses of substrates

The analysis of OMWW and CW samples involved a comprehensive assessment of several physicochemical parameters. The pH of the samples was measured using a pH-meter (HANNA instruments, HI 2210–02, Portugal). Electrical conductivity (EC) was measured using a conductivity-meter (HANNA instruments, HI 9033, Portugal). Total suspended solids (TSS) were quantified by filtering the samples through Whatman membrane filters (nylon pore, size 0.45 µm, diam. 47 mm) followed by drying the residue at 105 °C until reaching a constant weight. Total solids (TS) were determined by oven drying 10 mL of the samples in an oven at 105 °C for 24 h, in accordance with the APHA standard method (APHA 1998). Total ash content was assessed by incinerating the TS at 550 °C for 6 h. Total Kjeldahl nitrogen (TKN) analysis involved mineralization and distillation. Specifically, 5 mL of OMWW was digested with a Kjeldahl catalyst tablet (0.5 g) and 10 mL of H_2SO_4 (96%) at 400 °C for 2 h. After cooling, 100 mL of distilled water and 50 mL of NaOH solution (40 g L^{-1}) were added before distillation, following AOAC guidelines (AOAC 2000). Chemical oxygen demand (COD) was determined following the Standard Methods for the Examination of Water and Wastewater (APHA 1998). Wastewater samples were diluted up to 10^3 times, and 2 mL of diluted OMWW were digested using sulfuric acid, potassium dichromate, and mercuric sulfate in a COD reactor at 150 °C for 120 min. COD concentration was measured calorimetrically at 600 nm. Total lipid content was assessed using the Soxhlet method with hexane as the solvent (AOAC 2000). Total sugar content was estimated according to the method outlined by Dubois et al. (1956), involving incubation of the samples with phenol and concentrated sulfuric acid at 100 °C for 5 min, and absorbance measurement at 490 nm. Reducing sugars were analyzed following the method described by Miller (1959), wherein samples were incubated with DNS reagent in a boiling water bath, followed by absorbance measurement at 540 nm. The concentration of reducing sugars (as glucose equivalent in g L^{-1}) was determined from a pre-stablished standard curve. Polyphenolic compounds (PC) were extracted from OMWW and purified with ethyl acetate according to the method of Macheix et al. (1990) and their concentration was assessed using Folin–Ciocalteu reagent, followed by absorbance measurement at 760 nm. The concentration of polyphenols (as gallic acid equivalent in g L^{-1}) was determined from a pre-established standard curve. All tests were carried out in triplicate at least.

Biomass concentration determination

Flasks were periodically removed from the rotary thermostatic shaker and cell biomass was harvested by centrifugation of the culture medium at 6000 rpm for 30 min. The pellets were then washed with distilled water and dried at 40 °C until a constant weight. The dried biomass was kept in the dark at −20 °C until analysis.

Reducing sugar uptake

The reducing sugar uptake in the cultivation spent media was calculated as follows (Eq. 1):

$$Reducing\ sugar\ uptake\,(\%) = \frac{(Ri - Rf)}{Ri} x100 \qquad (1)$$

where R_i is the initial reducing sugar concentration before cultivation and R_f is the reducing sugar concentration after cultivation. The test was carried out in triplicate.

Characterization of the media post cultivation

COD removal rate

COD rate from the spent cultivation media was calculated using the following formula (Eq. 2):

$$COD\ removal\,(\%) = \frac{(Pi - Pf)}{Pi} x100 \qquad (2)$$

where P_i is the initial COD concentration before cultivation and P_f is the final COD concentration (after cultivation). The test was carried out in triplicate. To validate the degradation capacity of the studied strain, control cultures were established without inoculating *R. glutinis*. These control experiments were conducted under identical cultivation conditions, serving as a comparative baseline for evaluating the effectiveness of COD removal by the strain.

Polyphenols removal rate

Polyphenols removal rate from the spent cultivation media was calculated using the following formula (Eq. 3):

$$Polyphenols\ removal\ (\%) = \frac{(Pi - Pf)}{Pi} x100 \quad (3)$$

where P_i is the initial polyphenol concentration before cultivation and P_f is the final polyphenol concentration (after cultivation). The test was carried out in triplicate. To validate the degradation capacity of the studied strain, control cultures were established without inoculating *R. glutinis*. These control experiments were conducted under identical cultivation conditions, serving as a comparative baseline for evaluating the effectiveness of polyphenol removal by the strain.

HPCL -phenolic compounds profile

Phenolic compounds profile in the spent media was determined by the method of Susamci et al. (2017) with some modifications. An aliquot of 1.5 mL of polyphenol extract was centrifuged for 5 min at 9000 rpm and subsequently filtered into another tube through a 0.22 µm filter. An aliquot of 0.25 mL of the supernatant was diluted with 0.5 mL of DMSO and 0.25 mL of 0.2 mM syringic acid prepared in DMSO (internal standard). An aliquot of 20 µL of the mixture was injected into the High-Performance Liquid Chromatography (HPLC). The HPLC system consisted of 1260 vial sampler (Agilent Technologies), 1100 (Quat. Pump) (Hewlett Packard), 1200 diode array detector (Agilent Technologies). A Phenomenex Luna 5 µm C18 100 A 250 × 4.6 mm column was also used. Separation was achieved using an elution gradient with an initial composition of 90% (v/v) water (pH adjusted to 2.7 with phosphoric acid) and 10% (v/v) methanol. The concentration of the second solvent was increased to 30, 40, 50, 60, 70 and 100% (v/v) over a total of 68 min. A flow rate of 1 mL min^{-1} and a temperature of 35 ºC were also used. Co-injections with commercial standards including hydroxytyrosol, tyrosol, oleuropein, caffeic acid, luteolin, apigenin and syringic acid were performed to validate compound identity. Calibration curves for the analyzed compounds were constructed by injecting 10 µL of standard solutions at five different concentrations. The chromatograms were recorded at 280 and 330 nm for phenolic compounds. The concentration of phenolic compounds was expressed as mg kg^{-1} of extract. The test was carried out in triplicate.

Biomass composition determination

Protein content determination

Protein content was determined using the Micro-Kjeldahl method following the method described in Ogg (1960) with slight modifications. Briefly, 15 mg of yeast sample was placed in a micro-Kjeldahl digestion flask, followed by the addition of 2 mL of concentrated sulfuric acid and an appropriate amount of the catalyst. The mixture was heated at 360–410°C until a clear solution was obtained. After cooling, the digest was diluted with distilled water and transferred to a distillation apparatus. A solution of NaOH (40%) was added to release ammonia gas (NH_3), which was distilled and absorbed in a boric acid solution (4%). The ammonia absorbed in boric acid was titrated using 0.1 N H_2SO_4, with a mixed indicator to determine the endpoint. Protein content was determined by calculating the total nitrogen from the volume of acid consumed, applying blank correction, and multiplying by a conversion factor of 6.25.

Lipid content determination

An aliquot of 5 mL of a methanol/water/HCl (30:3:1, v/v/v) mixture was added to 30 mg of dried biomass, into clean, screw-capped glass test tubes and incubated at 55 °C for 6 h (Rezanka et al. 2003). A total of 15 mL of cold water–hexane (2:1, v/v) mixture was added to each sample and homogenized for 20 s. The hexane layer was filtered and concentrated to dryness under a stream of nitrogen. This extraction was repeated three times using 5 mL chloroform on the residue. The extract was filtered and concentrated to dryness under a stream of nitrogen. All the obtained dried extracts were combined and dissolved in 1 mL of hexane–chloroform (1:1, v/v) mixture and then evaporated under a nitrogen stream. The total lipid content was estimated gravimetrically and expressed in % DW. The test was carried out in triplicate.

Fatty acid profile

Trans-esterification of lipids was performed in a two-stage reaction (to prevent trans-isomer formation) using $CH_3O^-Na^+$ and CH_3OH/HCl following to the AFNOR method (1984). Fatty acid methyl esters (FAME) were analyzed in a Shimadzu, GC 17A Gas Chromatography equipped with an FID detector and a capillary column (50 m × 0.32 mm, 0.5 mm, PERICHROM Sarl, France). Nitrogen was used as carrier gas, at a flow rate of 1.13 mL min^{-1}.

The oven temperature was started from 100 °C, increased to 150 °C at a rate of 30 °C min^{-1} and held for 5 min. The temperature was then increased to 190 °C at a rate of 10 °C min^{-1} and held for 14 min before being increased (at 5 °C min^{-1}) to 255 °C and held for 10 min. The injector and the detector temperature were adjusted at 255 and 270 °C, respectively. The FAME peaks were identified by reference to standards. The test was carried out in duplicate.

Total carotenoids content determination

To quantify carotenoid content, a solid-to-liquid ratio of 1:5 (w/v) was employed using dimethyl sulfoxide (DMSO) as the solvent. Carotenoids were extracted using a mortar to disrupt the yeast cells, and extraction was repeated multiple times to achieve maximal recovery of intracellular carotenoids until the biomass appeared gray. Following extraction, samples were centrifuged at 7,000 rpm for 5 min to separate solid particles from the carotenoid extract. Subsequently, 2 mL of acetone, petroleum ether, and 20% sodium chloride solution were added to the extract. The resulting samples were vigorously shaken for 1 h and centrifuged at 3,500 rpm for 5 min to facilitate phase separation. The colored ether phase was then carefully transferred to a cuvette, and its absorbance was measured spectrophotometrically at 490 nm. The total carotenoid content (µg/g dry mass) was calculated using the following equation (Eq. 4) (Cheng et al. 2016):

$$\text{Total carotenoids}\left(\frac{\mu g}{g}\text{of biomass}\right)=\frac{\text{Amax . D . V}}{\text{E . W}} \quad (4)$$

$A_{max:}$ Absorbance at 490 nm.
D: Sample dilution ratio.
V: Volume of extraction solvent (mL).
E: Extinction coefficient of total carotenoids (0.16).
W: Dry weight of yeast biomass (g).

Total carotenoid extract production and characterization

R. glutinis biomass production was carried out in a 10 -L reactor using the OC50 medium, inoculated at 10% (v/v). The culture was maintained at 30 °C with continuous stirring at 150 rpm. After 8 days of cultivation, the biomass was harvested by centrifugation at 6000 rpm for 30 min and the pellet was thoroughly washed and dried in an oven at 40 °C. Total carotenoids were then extracted from the dried biomass using DMSO, following the method described above. The extracted total carotenoids (TCE) were stored in the dark at –20 °C until further analysis.

FTIR and thermostability properties

To investigate the structural properties of the total carotenoid extract (TCE), Fourier-transform infrared (FTIR) spectroscopy was performed using approximately 0.5 mg of sample. The ample was analyzed with 32 scans per minute and a resolution of 4 cm^{-1} in the wavenumbers region varying from 400 cm^{-1} to 4000 cm^{-1} using a Nicolet FTIR spectrometer equipped with an attenuated total reflection (ATR) accessory.

The thermostability of TCE was assessed by exposing TCE (50 µg/mL in DMSO) to various temperatures: 40, 60, 80, and 120 °C. Samples were collected at 0, 30, 60, 90, 120, 150, 180-, 210-, 240- and 270-min. Thermal degradation was monitored by measuring the decrease in absorbance at 490 nm, corresponding to the carotenoid peak. TCE stability was expressed as the relative remaining concentration (R_{TCE}, %), calculated using the equation:

$$R_{TCE}\% = \left(A_{TCE\ (f)}/A_{TCE\ (i)}\right) \times 100 \quad (5)$$

where $A_{TCE\ (f)}$ is the absorbance at 490 nm after heat treatment, $A_{TCE\ (i)}$ is the initial absorbance at 490 nm.

Antioxidant properties

DPPH radical-scavenging assay

The DPPH radical-scavenging activity was determined according to the method of Bersuder et al. (1998), with slight modifications using TCE from *R. glutinis* solution (6.43 to 102.8 µg mL^{-1}). BHA was used as a positive control. The samples were incubated for 60 min at room temperature. DPPH radical-scavenging activity was calculated as follows:

$$\text{DPPH radical} - \text{scavenging activity}\ (\%) = \left(A_{Control} + \left(A_{Blank} - A_{Sample}\right)/A_{Control}\right) \times 100 \quad (6)$$

where $A_{Control}$: is the absorbance of the control (containing all reagents except the sample) at 517 nm, A_{Blank}: is the absorbance of the blank (containing all reagents except the DPPH solution) at 517 nm. A_{Sample} is the absorbance of the TCE solution at 517 nm. The test was carried out in triplicate.

Ferric reducing antioxidant power

The TCE (6.43 to 102.8 µg mL^{-1}) ability to reduce iron was determined according to the method of Yıldırım et al. (2001). BHA was used as a positive control. The absorbance

of the resulting solutions was measured at 700 nm after 10 min of incubation. The test was carried out in triplicate.

Antibacterial activity

Antibacterial activity of TCE (50 µg mL^{-1}) was tested using the well diffusion assay, following the method described by Vlietinck (1991), against two Gram-negative strains (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 15442) and two Gram-positive strains (*Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633). *S. aureus*, *E. coli* and *P. aeruginosa* were selected as foodborne and pathogenic bacteria commonly associated with food contamination and opportunistic infections (Kuznetsova et al. 2025; Phan et al. 2025). *Bacillus subtilis* was included as a non-pathogenic Gram-positive model with probiotic relevance for feed applications (Olmos et al. 2020). Briefly, a 18 h-culture suspension of each indicator strain was adjusted to a concentration of 10^6 CFU mL^{-1} was uniformly spread on sterile Mueller Hinton agar plates. Wells of 6 mm diameter were prepared on each plate with a sterile cork borer. An aliquot of 75 µL of TCE solution was then loaded in each well and allowed to diffuse at 4 °C for 2 h. DMSO was used as a control. Subsequently, plates were kept at 37 °C for 18 h and the diameter of the growth inhibition zone (mm) around the well was measured in duplicate.

Statistical analysis

All experiments were conducted at least in duplicate. Data were expressed as means ± SD (standard deviation) and analyzed using SPSS version 19.0 for Windows professional edition (SPSSInc., Chicago, USA). Analysis of variance (ANOVA) was then performed to estimate the statistical significance at a 5% probability level ($p = 0.05$).

Results and discussion

Physicochemical characterization of substrates

The composition of the OMWW and CW employed in the present study is indicated in Table 2. Clear differences were found in total and reducing sugars, nitrogen, lipid contents, COD and phenolic load. CW contained the highest total sugars (48.23 ± 1.52 g L-1) and nitrogen (1.7 g L-1), whereas OMWW had more reducing sugars (34.53 ± 2.17 g L-1) and minimal nitrogen (0.01 ± 0.003 g L-1). These complementary profiles highlight their potential as mixed substrates, making the C/N ratio a key parameter for simultaneous microbial growth and metabolite production (Lopes et al. 2020). OMWW showed a lower pH (4.48) than CW (5.42), mainly due to organic acids and phenolics (Achak et al. 2019). Both values are below the optimal yeast growth pH (5.5–6.0) (Mussagy et al. 2020; Taskin et al. 2016; Allahkarami et al. 2021), indicating the need for adjustment before use. COD reached 129.9 ± 23.05 g L-1 in OMWW and 54.85 ± 6.01 g L-1 in CW, confirming high organic loads. OMWW also contained 6.33 ± 0.49 g L-1 phenolic compounds, within the 0.5–12 g L-1 reported in the literature (Keskin et al. 2023; Boutafda et al. 2019; El Feky et al. 2019; Arous et al. 2016). Such parameters vary with origin, cultivar, ripeness, milling and extraction methods (Ben Sassi et al. 2006; Souilem et al. 2017; Fleyfel et al. 2022; Khelouf et al. 2023; Issa et al. 2023), and our results align with these ranges.

Table 2 Physico-chemical characterization of OMWW and CW

Parameters	OMWW	CW
pH	4.48 ± 0.06	5.42 ± 0.04
EC (mS/cm)	12.59 ± 0.1	3.83 ± 0.2
TS (g L^{-1})	111.60 ± 4.3	39.06 ± 1.35
COD (g L^{-1})	129.90 ± 13.05	54.85 ± 6.01
Total ash (g L^{-1})	14.67 ± 3.06	3.849 ± 0.37
TKN (g L^{-1})	0.01 ± 0.00	1.70 ± 0.20
Total Sugar (g L^{-1})	42.56 ± 1.07	48.23 ± 1.52
Reducing Sugar (g L^{-1})	34.53 ± 2.17	23.12 ± 1.10
Lipids (g L^{-1})	4.38 ± 0.23	1.28 ± 0.12
Polyphenols (g L^{-1})	6.33 ± 0.49	/
Polyphenols profile (mg kg^{-1}):		
• Hydroxytyrosol	230.37 ± 0.77	/
• Tyrosol	98.68 ± 3.44	/
• Oleuropein	53.85 ± 1.14	/
• Caffeic acid	16.64 ± 0.32	/
• Luteolin	37.88 ± 0.39	/
• Apigenin	34.55 ± 0.43	/

The pH values of OMWW and CW also diverged; OMWW exhibited the lowest pH value (4.48) due to the presence of organic acids resulting from auto-oxidation reactions and phenolic compounds polymerisation (Achak et al. 2019), while CW displayed a relatively higher pH value (5.42). However, both pH values fall below the optimal pH value for yeast growth ranging between 5.5 to 6.0 (Mussagy et al. 2020; Taskin et al. 2016; Allahkarami et al. 2021), necessitating pH adjustment before utilizing OMWW and CW as growth substrates. The COD values in OMWW and CW were estimated at 129.9 ± 23.05 g L^{-1} and 54.85 ± 6.01 g L^{-1}, respectively, confirming a significant organic load in both wastewaters. Additionally, OMWW exhibited a high phenolic compound (PC) concentration of 6.33 ± 0.49 g L^{-1}, which falls within the range reported in the literature (0.5 to 12 g L^{-1}) (Keskin et al. 2023; Boutafda et al. 2019; El Feky et al. 2019; Arous et al. 2016). It is important to note that both PC and COD values can vary considerably depending on several factors, including geographical origin,

olive cultivar, fruit ripeness, milling method, and extraction technique, as documented in previous studies (Ben Sassi et al. 2006; Souilem et al. 2017; Fleyfel et al. 2022; Khelouf et al. 2023; Issa et al. 2023). The values obtained in the present study are therefore consistent with the variability observed across different contexts.

Growth of *R. glutinis* on OMWW and CW mixtures—based media

To evaluate the effect of C/N ratio on *R. glutinis* growth, cultures were run for 10 days in media with varying OMWW/CW mixtures (OC25–OC100; pH 5.7). As shown in Fig. 1, the best growth occurred in OC25 and OC50 (C/N 20:1 and 35:1), reaching similar maxima of 5.16 ± 0.18 and 5.19 ± 0.11 g L^{-1} after 3—5 days (Table 3). OC75 showed slower accumulation, peaking at 2.67 ± 0.13 g L-1 around day 8. In contrast, OC100 (100% OMWW) strongly inhibited growth, yielding only 0.58 ± 0.06 g L-1 by day 10, highlighting the unsuitability of undiluted OMWW. Indeed, this result could be explained by the elevated polyphenols concentration in OC100 (6.33 g L^{-1}) that may have exerted an inhibitory effect on yeast growth. A similar trend was reported by Ghilardi et al. (2020), who observed growth inhibition of *R. mucilaginosa* when cultured in a semi-liquid olive mill wastewater medium referred to as "aqueous extract of alperujo", at 30% w/v, containing 7.6 g L^{-1} of polyphenols. Moreover, the media that supported the highest *R. glutinis* growth, OC25 and OC50, also exhibited the highest final pH values, reaching 9.05 ± 0.10 and 8.97 ± 0.06, respectively (Table 3).

This pH increase is attributed to the yeast's metabolic activity, particularly the assimilation of organic acids and the deamination of amino acids, both of which lead to the alkalinization of the medium (Hafidi et al. 2005). Similar results were observed with *R. glutinis* grown on potato wastes (Kot et al. 2017), olive mill wastewater (Karakaya et al. 2012), and with a strain of *R. mucilaginosa* used to treat olive mill wastewater (Jarboui et al. 2012) and alperujo (Ghilardi et al. 2020).

Furthermore, the present findings demonstrate that the OC25 and OC50 media, characterized by relatively low carbon-to-nitrogen (C/N) ratios of 20:1 and 35:1, respectively, significantly promoted the growth of *R. glutinis*, as

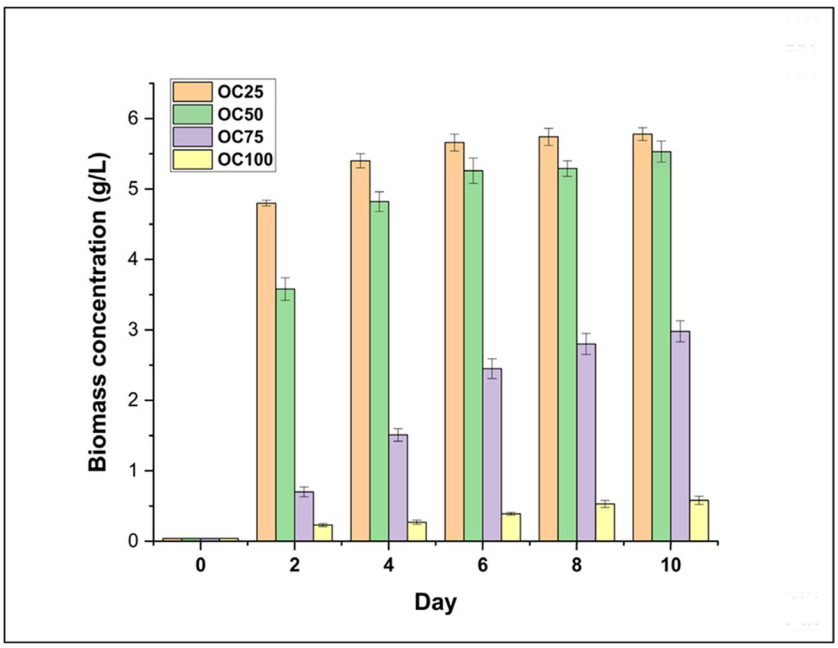


Fig. 1 Biomass concentration of *R. glutinis* grown in OC25, OC50, OC75 and OC100 at 150 rpm and 30 °C (pH 5.7) over 10 days. Error bars presenting the standard deviation (n = 3)

Table 3 Growth parameters of *R. glutinis* cultivated in different OMWW-CW mixtures at 150 rpm and 30 °C (pH 5.7)

Code	C/N ratio	Polyphenols (g L^{-1})	End log phase (Day)	Max. biomass at end log phase (g L^{-1})	Max. biomass at day 10 (g L^{-1})	Final pH (Day 10)
OC25	20	1.83	3	$5.16 \pm 0.58^{a*}$	$5.78 \pm 0.09^{a*}$	9.05 ± 0.10^{a}
OC50	35	2.62	5	$5.19 \pm 0.31^{a*}$	$5.53 \pm 0.17^{a*}$	8.97 ± 0.06^{a}
OC75	72	4.41	8	$2.67 \pm 0.13^{a**}$	$2.98 \pm 0.35^{a**}$	6.87 ± 0.03^{b}
OC100	287.5	6.33	NG	NG	$0.58 \pm 0.05^{***}$	5.90 ± 0.02^{c}

Different letters in the same line indicate significant difference ($p<0.05$). Different stars in the same column indicate significant difference ($p<0.05$). (n=3). NG: No growth

evidenced by the elevated biomass concentrations achieved within the cultivation period. These results are consistent with previous studies on various *Rhodotorula* species, in which optimal biomass production was achieved at C/N ratios ranging from 12 to 20 (Taskin et al. 2016; Ribeiro et al. 2019; Tkáčová et al. 2018).

In addition, the strain appeared to exhibit tolerance to elevated polyphenol concentrations. Cultivation in the IC50 medium, containing a relatively high polyphenol level (2.6 g L^{-1}), resulted in a biomass yield of 5.19 ± 0.3 g L^{-1} within five days. When compared to previous studies, similar biomass yields (approximately 5.70 g L^{-1}) were only achieved after six days of cultivation in water-diluted OMWW, which contained a significantly lower polyphenol concentration (approximately 0.5 g L^{-1}) and was supplemented with urea as a nitrogen source (0.5 g L^{-1}) (Karakaya et al. 2012; Boutafda et al. 2019; Ghilardi et al. 2022; Keskin et al. 2023).

To the best of our knowledge, the combination of OMWW and CW as a culture medium for *R. glutinis* cultivation has never been previously explored. In the present study, the enhanced growth performance observed in this mixed substrate may be attributed to the nutrient-rich composition of CW, which provides readily assimilable lactose, peptides, essential minerals, and vitamins, components known to stimulate cell proliferation and previously associated with improved yeast productivity under similar conditions (Prazeres et al. 2012; Braunwald et al. 2013; Arous et al. 2016). Moreover, the presence of CW may mitigate the inhibitory effects of the high polyphenol content in OMWW by supplying metabolic precursors and buffering agents that help maintain intracellular homeostasis, thereby enabling *R. glutinis* to thrive under stress conditions. Collectively, these findings highlight the dual role of CW as both a nitrogen-rich substrate and a protective matrix, enhancing yeast viability and productivity even in challenging environments.

Reducing sugar uptake

The reducing sugar (RS) uptake by *R. glutinis* cultivated on varying mixtures of OMWW and CW (OC25, OC50, OC75 and OC100, pH 5.7) at 150 rpm and 30 °C, over 10-day period and the results are presented in Fig. 2. The OC25 and OC50 media exhibited significantly higher RS uptake, reaching the values of 56.21 ± 0.87% and 51.96 ± 1.91%, respectively, by days 4 and 6, corresponding to the end of the exponential growth phase of *R. glutinis*. No significant increase in RS uptake was observed over days 4 and 6. The favorable C/N ratios facilitated optimal sugar assimilation, which also explains the observed rapid cell proliferation, with the nitrogen supply from CW sustained growth and enhanced metabolic activity, resulting in substantial sugar consumption (Cai et al. 2022). In contrast, the uptake of RS was lower in OC75 and OC100. This reduced uptake is probably due to the elevated levels of polyphenols in those media, which may inhibit key enzymes involved in the metabolism of disaccharides and oligosaccharides. Indeed, various natural polyphenols, including flavonoids and phenolic alcohols, are known to act as potent enzyme inhibitors, with reported IC_{50} values as low as 0.6 mg L^{-1} (Rasouli et al. 2017; Renda et al. 2018; Lim et al. 2019), thereby limiting sugar assimilation by the yeast. It is important to highlight that the sugar uptake patterns align with the trends observed in biomass production and pH under lower C/N conditions.

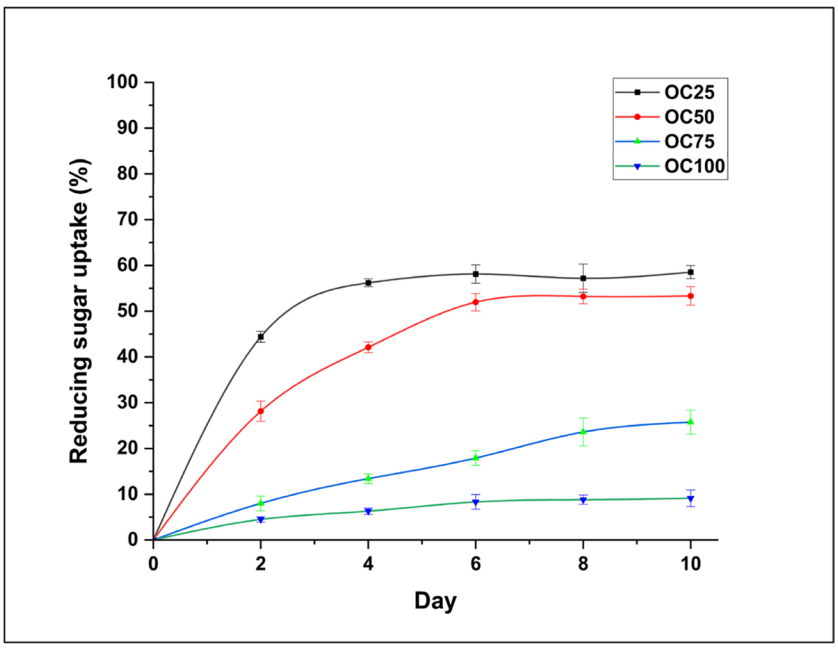


Fig. 2 Reducing sugar uptake by *R. glutinis* cultivated in OC25, OC50, 0C75 and OC100 at 150 rpm and 30 °C (pH 5.7) over 10 days. Error bars presenting the standard deviation (n = 3)

COD removal

Figure 3 shows the COD removal kinetics by *R. glutinis* in different media. In OC100, only 1.84±0.06% COD removal was achieved after 10 days versus 0.24±0.03% in the control (Table 4), reflecting inhibition by high phenolic content and low nitrogen. Conversely, OC25 and OC50 showed much higher removals: within 2 days 10.42±0.18% and 3.73±0.043%, and by day 10 peaks of 55.92±0.88% and 41.19±0.25%, compared with 1.79±0.62% and 1.25±0.14% in the controls. These results confirm the critical role of *R. glutinis* in the biodegradation process and highlight the synergistic effect of co-substrate enrichment on depollution performance and the yeast's capacity to metabolize biodegradable organic fractions, such as reducing sugars, soluble proteins, and simple phenolics.

The observed trends are consistent with previous findings. Fakharedine et al. (2011) reported an 86.45% COD reduction after 20 days of aerobic OMWW treatment using *R. glutinis*, while Karakaya et al. (2012) achieved approximately 80% COD removal in 14 days under similar conditions. Jarboui et al. (2012) further demonstrated the versatility of the genus, with *R. mucilaginosa* removing between 38% and 56.91% COD over a 6-day period, depending on initial substrate concentrations.

Polyphenols removal

Removal efficiency

As shown in Fig. 4, *R. glutinis* exhibited a progressive increase in polyphenol removal efficiency from day 0 to day 10 in all media, except OC100. By day 10, removal efficiencies reached 58.05±1.10% for OC25, 57.37±1.56% for OC50, and 22.91±2.14% for OC75. The ability of yeasts in general, and *Rhodotorula* strains in particular, to degrade polyphenols is attributed to a multifactorial enzymatic system involving both oxidative and non-oxidative pathways. *R. glutinis* is known to exhibit laccase-like and peroxidase activities, especially under stress or in the presence of complex phenolic mixtures (Katayama-Hirayama et al. 1991; Cadete et al. 2017; Mtui 2012). These enzymes facilitate the oxidative breakdown of polyphenolic rings into simpler and less toxic compounds. In parallel, reductases and dehydrogenases contribute to detoxifying intermediate metabolites, potentially enhancing overall bioremediation performance (Liu et al. 2025). Moreover, under polyphenolic stress, *R. glutinis* induces antioxidant enzymes and other stress-related mechanisms, further supporting its degradation capabilities (Salar et al. 2012).

In contrast, OC100 exhibited a significantly lower removal efficiency of just 1.58±0.08%. This finding supports the hypothesis that high polyphenol concentrations in

Table 4 Polyphenols removal rate and COD removal in cultivation media without *R. glutinis* inoculation at 150 rpm and 30 °C (pH 5.7) on day 10

	OC25	OC50	OC75	OC100
PC removal rate (%)	2.42±0.12^{a}	2.27±0.11^{b}	1.33±0.04^{c}	0.89±0.13^{d}
COD removal (%)	1.79±0.62^{a}	1.25±0.14^{a}	0.46±0.19^{b}	0.24±0.03^{c}

Different letters in the same line indicate significant difference ($p<0.05$)

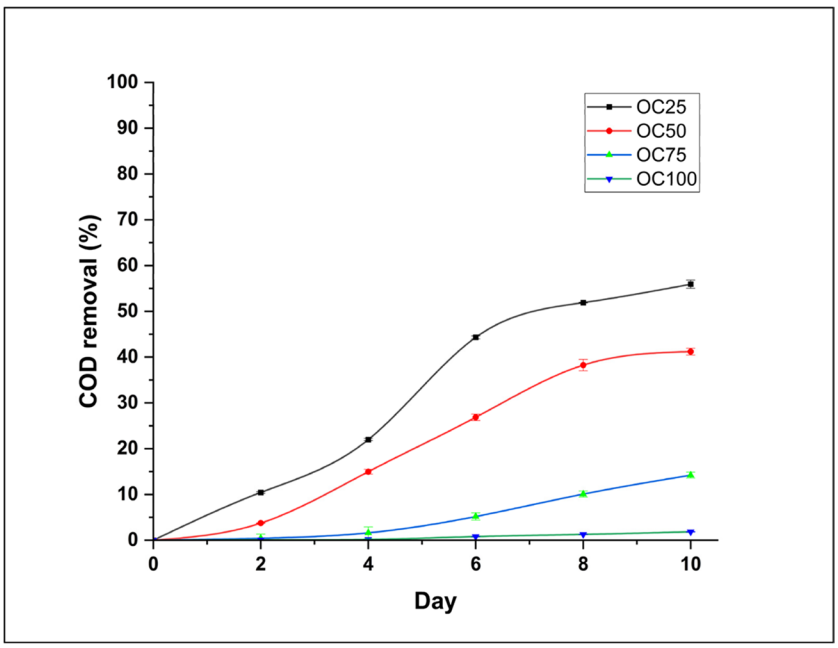


Fig. 3 COD removal by *R. glutinis* cultivated in OC25, OC50, 0C75 and OC100 at 150 rpm and 30 °C (pH 5.7) over 10 days. Error bars presenting the standard deviation (n=3)

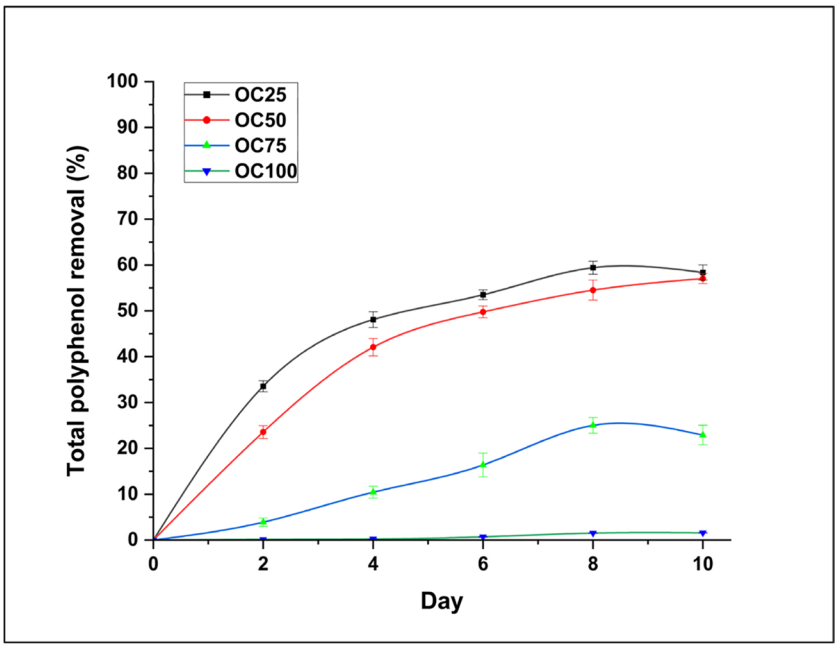


Fig. 4 Polyphenols removal by *R. glutinis* cultivated in OC25, OC50, 0C75 and OC100 at 150 rpm and 30 °C (pH 5.7) over 10 days. Error bars presenting the standard deviation (n = 3)

OMWW, well-documented for their antimicrobial activity, can inhibit yeast growth, disrupt enzymatic activity, and limit biotransformation processes (Alrowais et al. 2023). The observed gradient in removal efficiency across treatments highlights the importance of OMWW dilution in optimizing bioremediation performance, likely reflecting a balance between substrate availability and toxicity. In this context, the bioremediation role of the studied *R. glutinis* was further confirmed through control cultivations performed under similar conditions in OC25, OC50, OC75, and OC100, but without strain inoculation. In the absence of *R. glutinis*, removal rates were significantly lower, recorded at 2.42 ± 0.12%, 2.27 ± 0.11%, 1.33 ± 0.04% and 0.89 ± 0.13% for OC25, OC50, OC75 and OC100, respectively, as shown in Table 4.

These low values therefore confirm that the polyphenol degradation observed in the inoculated.

cultures were largely due to the metabolic activity of *R. glutinis*, rather than spontaneous degradation or abiotic factors. The performance observed in this study is consistent with previous reports highlighting the sensitivity of polyphenol removal efficiency to the initial concentration of phenolic compounds in OMWW.

Several studies have demonstrated that increasing polyphenol content can significantly reduce the bioremediation potential of yeast strains, including those from the *Rhodotorula* genus. In line with this, Karakaya et al. (2014) reported that batch biodegradation with interval feedings was more effective during the early stages of operation, but prolonged exposure led to decreased dephenolization due to phenolic accumulation inhibiting cell growth, even though the overall removal was higher than in simple batch cultures.

These findings are in line with previous studies demonstrating the inhibitory effect of high phenolic concentrations on yeast-mediated bioremediation. Boutafda et al. (2019) reported that *R. mucilaginosa* achieved a polyphenol removal efficiency of 96.98% in 25% diluted OMWW, which progressively declined to 58.56%, 16.59%, and 11.86% in 50%, 75%, and 100% dilutions, respectively, over a one-month cultivation period under static conditions. This trend clearly supports the notion that phenolic toxicity at higher concentrations suppresses yeast activity and enzyme performance. Comparable results were equally observed by Jarbaoui et al. (2012), who recorded polyphenol removal rates of 34.81%, 27.89%, and just 5.84% at increasing initial polyphenol concentrations of 0.83, 1.66, and 3.3 g L^{-1}, respectively, after six days of *R. mucilaginosa*. In a related study, *R. glutinis* demonstrated a removal efficiency of up to 83% from OMWW containing 0.9 g L^{-1} polyphenols when supplemented with 1 g L^{-1} yeast extract as a nitrogen source, following 19 days of aerobic cultivation at 150 rpm (Karakaya 2012). Additionally, Martínez-García et al. (2007) demonstrated that diluting OMWW with cheese whey (CW) in a 75:25 (v/v) ratio enhanced phenol removal by *Candida tropicalis*, achieving a 54% reduction. Overall, these findings underscore the critical role of OMWW dilution, initial polyphenol concentration and nutrient supplementation in determining the effectiveness of *R. glutinis* bioremediation processes.

Polyphenols profile post cultivation process

Following the assessment of overall polyphenol removal efficiency, the polyphenolic profile of the spent media was

analyzed to investigate the degradation kinetics of individual compounds over time. Identification was performed by comparing their retention times with those of standard compounds and the data presenting the concentration variations of key polyphenols in OC100, OC75, OC50, and OC25 media, sampled at 2, 4, 6, 8, and 10 days are presented in Table 5.

Based on this analysis, six major polyphenolic compounds were identified in the spent cultivation media samples, namely hydroxytyrosol, tyrosol, caffeic acid, oleuropein, luteolin, and apigenin, detected at absorption wavelengths of 280 and 330 nm. Among these, hydroxytyrosol was the most abundant across all cultivation media, followed by tyrosol, while oleuropein and caffeic acid were also detected. These results align with previously reported data on OMWW profile (El Abbassi et al. 2012; Otero et al. 2021; Zahi et al. 2022), including studies focusing on Algerian OMWW (Ladhari et al. 2021; Gueboudji et al. 2022a, 2022b). However, no comprehensive or standardized polyphenolic composition of OMWW exists due to high variability influenced by olive cultivar, ripeness, storage and processing (Cuffaro et al. 2023).

Due to their documented toxicity to microorganisms, plants, and animals within the trophic chain, the biodegradation of hydroxytyrosol and tyrosol represents an essential step toward mitigating the environmental impact of OMWW (Fiorentino et al. 2003).

Both compounds were significantly degraded in all media ($p < 0.05$). In OC25 (C/N 20:1) and OC50 (C/N 35:1), hydroxytyrosol decreased by 96.03% and 88.79%, and tyrosol by 85.92% and 76.89% after 10 days (SI.1). OC75 (C/N 72:1) showed lower removal (41.24% and 41.66%). Under low C/N conditions, *R. glutinis* also degraded other polyphenols. Oleuropein was completely removed in OC25 by day 2, versus 53.12% in OC50 and 24.98% in OC75. Caffeic acid declined by 75.26%, 64.09% and 26.91%, respectively. Luteolin and apigenin, though present in lower amounts, were fully degraded within 6–8 days in OC25/OC50, but only partially removed (66.35% and 76.18%) in OC75.

These results revealed that *R. glutinis* exhibited significantly enhanced biodegradation activity in media with lower C/N ratios, particularly in OC25 and OC50. These conditions, characterized by higher nitrogen availability compared to OC75, likely supported increased metabolic activity and biomass proliferation, thereby facilitating the efficient degradation of key phenolics such as hydroxytyrosol and tyrosol. The complete removal of oleuropein in OC25 by day 2, along with the substantial reduction of caffeic acid under similar conditions, further highlights the ability of *R. glutinis* to metabolize both simple and complex polyphenolic compounds. This performance may be attributed to the increased activity of hydrolytic enzymes such as flavonoid C8-hydroxylase (F8H) involved in breaking down structurally complex polyphenols (Madej et al. 2012; Madej et al. 2014; Sordon et al. 2016; Dulak et al. 2022) Overall, these findings suggest that nitrogen-rich environments stimulate enzymatic pathways linked to detoxification and phenolic assimilation, reinforcing the potential of *R. glutinis*

Table 5 Polyphenolic profile of the spent cultivation media of *R. glutinis* grown in different OMWW-CW mixtures at 150 rpm and 30 °C (Ph 5.7) over 10 days

		Hydroxytyrosol	Tyrosol	Oleuropein	Caffeic acid	Luteolin	Apigenin
(mg kg^{-1})							
OC75 (C/N 72:1)	0	197.52 ± 1.84^{a}	54.41 ± 0.93^{a}	47.65 ± 1.22^{a}	9.44 ± 0.06^{a}	11.95 ± 0.24^{a}	10.41 ± 0.10^{a}
	2	180.43 ± 0.53^{b}	48.02 ± 0.62^{b}	46.29 ± 0.67^{a}	8.13 ± 0.82^{b}	11.28 ± 0.12^{b}	8.78 ± 0.34^{b}
	4	168.64 ± 2.35^{c}	35.48 ± 0.24^{c}	39.36 ± 0.23^{b}	8.79 ± 0.06^{b}	10.57 ± 0.63^{c}	7.35 ± 0.66^{c}
	6	146.98 ± 0.24^{d}	33.89 ± 0.05^{d}	36.36 ± 0.30^{c}	7.10 ± 0.17^{c}	9.71 ± 0.02^{d}	7.09 ± 0.08^{c}
	8	116.12 ± 0.06^{e}	32.19 ± 0.51^{e}	35.71 ± 0.84^{c}	6.95 ± 0.04^{c}	8.48 ± 0.16^{e}	6.40 ± 0.32^{d}
	10	115.96 ± 0.16^{f}	31.25 ± 1.22^{e}	35.74 ± 0.50^{c}	6.90 ± 0.17^{c}	8.02 ± 0.02^{f}	6.48 ± 0.08^{d}
OC50 (C/N 35:1)	0	114.63 ± 2.49^{a}	30.60 ± 0.52^{a}	26.04 ± 0.69^{a}	6.99 ± 0.03^{a}	6.72 ± 0.13^{a}	5.86 ± 0.06^{a}
	2	60.09 ± 1.20^{b}	15.76 ± 0.35^{b}	27.64 ± 1.60^{a}	5.31 ± 0.46^{b}	5.78 ± 0.07^{b}	4.94 ± 0.19^{b}
	4	38.61 ± 1.32^{c}	11.15 ± 0.14^{c}	17.95 ± 0.13^{b}	4.58 ± 0.03^{c}	4.82 ± 0.36^{c}	4.13 ± 0.36^{c}
	6	26.43 ± 0.13^{d}	8.31 ± 0.03^{d}	15.73 ± 0.17^{c}	4.00 ± 0.09^{d}	3.77 ± 0.01^{d}	3.14 ± 0.05^{d}
	8	19.07 ± 0.03^{e}	7.86 ± 0.29^{d}	12.58 ± 0.47^{d}	3.80 ± 0.02^{e}	0.00 ± 00^{e}	0.00 ± 00^{e}
	10	12.85 ± 0.09^{f}	7.07 ± 0.68^{d}	12.21 ± 0.28^{d}	2.51 ± 0.09^{f}	0.00 ± 00^{e}	0.00 ± 00^{e}
OC25 (C/N 20:1)	0	78.01 ± 1.05^{a}	18.54 ± 0.05^{a}	11.19 ± 1.06^{a}	3.72 ± 0.01^{a}	5.98 ± 1.07^{a}	4.79 ± 0.81^{a}
	2	51.13 ± 0.64^{b}	11.63 ± 0.21^{b}	0.00 ± 00^{b}	3.42 ± 0.06^{b}	4.24 ± 0.08^{b}	2.87 ± 0.07^{b}
	4	7.50 ± 0.40^{c}	3.90 ± 0.05^{c}	0.00 ± 00^{b}	2.88 ± 0.21^{c}	3.50 ± 0.34^{c}	2.89 ± 0.19^{b}
	6	6.93 ± 0.02^{d}	3.78 ± 0.23^{c}	0.00 ± 00^{b}	2.47 ± 0.05^{d}	3.09 ± 0.09^{c}	2.51 ± 0.12^{c}
	8	4.15 ± 0.09^{e}	3.68 ± 0.06^{c}	0.00 ± 00^{b}	2.16 ± 00^{e}	0.00 ± 00^{d}	0.00 ± 00^{d}
	10	3.09 ± 0.03^{e}	2.61 ± 0.01^{d}	0.00 ± 00^{b}	0.92 ± 0.06^{f}	0.00 ± 00^{d}	0.00 ± 00^{d}

Different letters in the same column for the same cultivation medium indicate significant difference (n=3) ($p<0.05$)

as a promising agent for the treatment of polyphenol-rich effluents such as olive mill wastewater.

Furthermore, existing studies highlight the significant polyphenol-degrading capabilities of the *Rhodotorula* genus. Karakaya et al. (2012) reported that *R. glutinis*, when cultivated in 25% OMWW supplemented with 2.27 g L^{-1} urea, achieved complete removal of hydroxytyrosol and tyrosol within just five days. Similarly, Boutafda et al. (2019) demonstrated that *R. mucilaginosa* achieved 97% total polyphenol removal after one month of treatment in 25% diluted OMWW, including the complete degradation of hydroxytyrosol, tyrosol, caffeic acid, *p*-coumaric acid, and oleuropein.

R. glutinis biomass composition

To investigate the effect of the C/N ratio on biomass composition, the *R. glutinis* yeast biomass harvested from OC25, OC50, and OC75 media were analyzed for their protein, total carotenoid, and lipid contents. Biomass obtained from the OC100 medium was excluded from compositional analysis due to its low yield (0.58 ± 0.06 g L^{-1} after 10 days of fermentation), which was insufficient for reliable assessment. The results are presented in Fig. 5.

Total protein content

The total protein content in *R. glutinis* biomass cultivated on OC25, OC50, and OC75 media (pH 5.7), under 150 rpm agitation at 30 °C over a 10-day period, is depicted in Fig. 5A. The data reveal a significant decline in protein content with increasing C/N ratios ($p < 0.05$). Indeed, in the OC25 medium (C/N 20:1), protein content was initially high reaching 73.56 ± 0.92% (DW) on day 2 and gradually decreased to 51.67 ± 0.37% (DW) by day 10. In contrast, a more significant decrease was observed in the OC50 (C/N 35:1) and OC75 (C/N 72:1) media, where protein content decreased from 64.04 ± 0.33% (DW) to 35.75 ± 1.11% (DW) and from 60.98 ± 0.21% (DW) to 45.09 ± 0.55% (DW) over the same period, respectively.

In the present study, a clear inverse relationship was observed between the C/N ratio and protein content. Indeed, the initial higher C/N ratios observed in the OC75 medium, resulted in lower protein levels compared to media with lower C/N ratio such as OC25, where nitrogen is more available. Furthermore, as fermentation progressed, nitrogen became significantly depleted leading to a progressive increase in the C/N ratio over time.

Given the essential role of nitrogen in protein biosynthesis, its depletion limited further protein accumulation and likely triggered a metabolic shift toward the synthesis of carbon-rich storage compounds such as lipids and carotenoids (El Bialy et al. 2011; Broach et al. 2012; Raita et al. 2022). These findings align with those reported by Kot et al. (2020), where protein content in *R. gracilis* grown in potato wastewater supplemented with glycerol under high C/N conditions, reached a maximum value of 51.6% (DW)

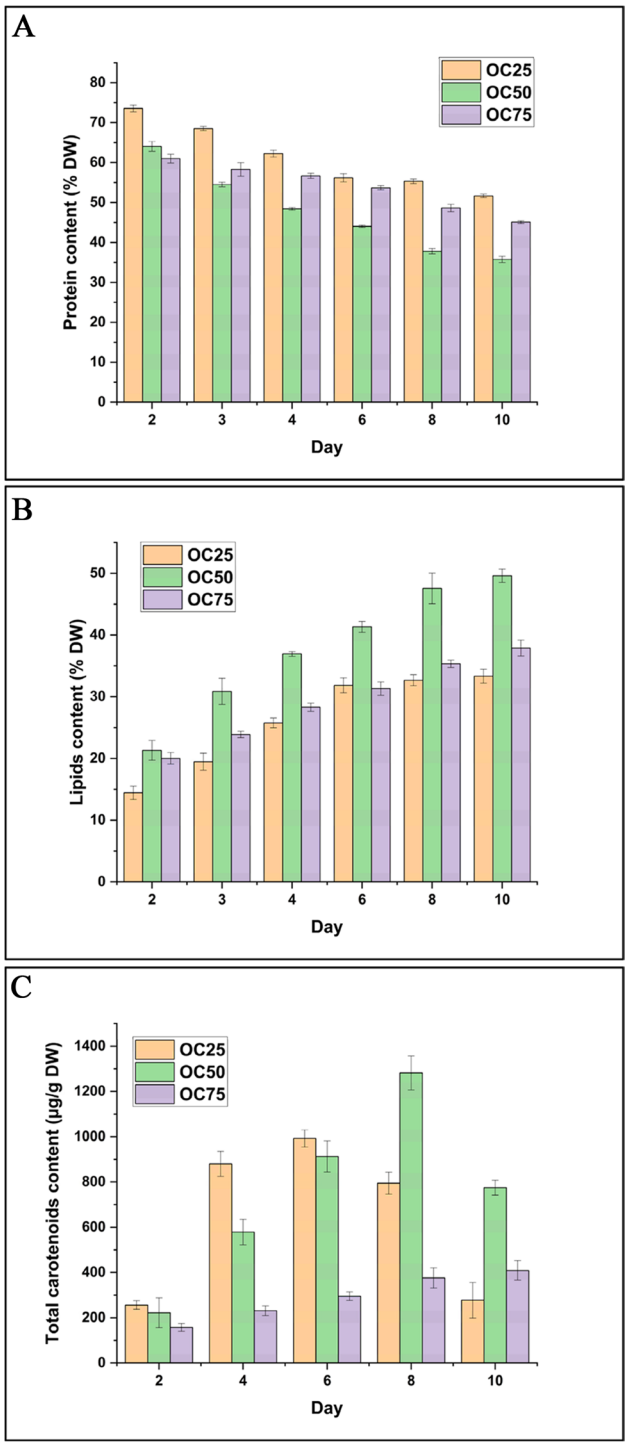


Fig. 5 Total protein (**A**), lipid (**B**) and total carotenoids (**C**) contents of *R. glutinis* cultivated in OC25, OC50, OC75 at 150 rpm and 30 °C (pH 5.7) over 10 days. Error bars presenting the standard deviation (n = 3)

after 2 days of cultivation time at 28 °C, followed by a significant decline by 6 days, reaching 30% (DW) while the lipid content increased by about 9% DW, suggesting a metabolic shift towards lipid accumulation.

Total lipids content and fatty acids profile

Figure 4B shows the total lipid content of *R. glutinis* grown on OC25, OC50 and OC75 (pH 5.7, 150 rpm, 30 °C, 10 days). Lipid content increased markedly over time in all media. In OC25 (C/N 20:1) it rose from 14.44 ± 1.1% to 31.83 ± 1.2% (DW) by day 6 and reached 33.33 ± 1.11% by day 10. In OC50 (C/N 35:1) it increased from 21.23 ± 1.59% on day 2 to 47.56 ± 2.5% on day 8. In OC75 (C/N 72:1) it rose from 20.02 ± 0.93% on day 2 to 37.91 ± 1.29% by day 10 ($p < 0.05$).

These results reveal the critical influence of the C/N ratio on lipid production in *R. glutinis* and highlight that the OC50 medium shows considerable potential as a lipogenic environment, yielding the highest lipid content after 8 days of cultivation.

Changes in lipid content during the cultivation period are commonly observed in oleaginous microorganisms and are influenced by various factors, including mainly growth phase stages, C/N ratio as well as nutrient availability (Fakas et al. 2007).

Several studies have shown that cell proliferation is the primary process when both carbon and nitrogen are present in the medium, while lipid synthesis is often initiated when the medium is overloaded with carbon and depleted of nitrogen sources (Weng et al. 2014; Abeln and Chuck 2021, Costa et al. 2024). Accordingly, the relatively lower C/N ratio in the OC25 medium likely provided sufficient nitrogen for growth and metabolic activities initially, leading to moderate lipid accumulation. As nitrogen became limiting after the exponential growth phase, lipid accumulation increased until a steady state was reached at stationary phase. Conversely, in the OC50 medium, the higher initial carbon availability and nitrogen limitation compared to the OC25 medium supported the higher lipid accumulation with lower growth. At this stage, the growth of yeast gradually slows due to nitrogen limitation, and carbon available in the substrate is used to accumulate lipids. Investigations of nitrogen-limited cultures have shown that a C/N ratio > 20 is required for oleaginous yeast, including *Rhodotorula* strains, in particular, to reach the maximum lipid production (Jiru et al. 2017; Lei et al. 2024). Indeed, under nitrogen-deficient conditions, the activity of isocitrate dehydrogenase is inhibited, leading to the accumulation of citrate in the mitochondria, which is then translocated to the cytoplasm, where it is released by adenosine triphosphate citrate lyase and the formation of acetyl-CoA, the precursor of fatty acids (Beopoulos et al. 2009; Chebbi et al. 2019; Ledesma-Amaro et al. 2016).

On the other hand, the higher C/N ratio in the OC75 medium (72:1) provided an abundance of carbon compared to nitrogen, promoting the observed continuous lipid accumulation over the cultivation period. However, the slower increase in lipid content compared to OC50 (35:1) suggests that a higher C/N ratio might not be as efficient for lipid biosynthesis. This may be due to higher initial substrate concentrations leading to growth inhibition in batch culture mode, resulting in lower cell growth rate and lipid yields (Abeln and Chuck 2021; Caporusso et al. 2021; Karamerou and Webb 2019; Valdes et al. 2021). A similar trend was observed in the study conducted by Filippousi et al. (2022) where lipid accumulation in *R. toruloides* under nitrogen-limited conditions (initial C/N molar ratio of 50—240), gradually decreased as the C/N ratio increased. Similarly, Keskin et al. (2023) reported that *R. glutinis* cultivated in olive mill wastewater (OMWW) containing 0.5 g L^{-1} of polyphenols, under different concentrations of urea (0, 0.5, and 1 g L^{-1}), exhibited the highest total lipid content of 15.12 ± 0.20% at a urea concentration of 0 g L^{-1}, after 6 days of cultivation. Lopes et al. (2020) reported an increase in lipid yield in the *R. toruloides* cultivated in raw glycerol at different range of C/N ratios (60, 80, 100, and 120). The highest lipid yield was observed at C/N ratio between 60 and 100. In this study, the maximum lipid content achieved was 47.56 ± 2.5% DW using a C/N ratio of 35 with a medium consisting of 50% OMWW and 50% CW. This lipid yield is competitive with lipid yield obtained with conventional and non-conventional carbon sources in similar growth conditions. For comparison, glucose-based media produced a lipid yield of 47.24% (Maza et al. 2020), while sucrose and fructose resulted in lower yields of 28% and 27.5%, respectively (Gong et al. 2019). Non-conventional substrates such as crude glycerol yielded a lipid content of 35.22% of total biomass (Saenge et al. 2011), and the combination of glycerol with used cooking oil achieved a lipid content of 46% (Yen et al. 2019). Additionally, sugarcane molasses provided a lipid content of 45.0% (Lakshmidevi et al. 2021), and residual glycerol from the biodiesel industry resulted in lipid yields ranging from 40.66% to 46.86% (Sineli et al. 2022).

Importantly, the OC50 and OC75 conditions, which exhibited the highest lipid contents, were also associated with the lowest protein contents, further confirming the metabolic shift from protein synthesis to lipid accumulation under nitrogen-limited conditions.

Following the assessment of lipid content, the fatty acid (FA) profiles of *R. glutinis* cultivated under the same media and conditions have been identified and the results are presented in Table 6. Results revealed that the major identified

Table 6 Fatty acids profile of *R. glutinis* grown in different OMWW-CW mixtures at 150 rpm and 30 °C (Ph 5.7) over 10 days

Cultivation Medium	Cultivation Day	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	Linolenic acid (C18:3)	N.I	UFAs	SFAs	C18:2/C18:3
%										
OC25 (20:1)	2	24.67 ± 0.22^{e}	15.09 ± 0.33^{e}	55.89 ± 0.93^{b}	4.46 ± 0.11^{a}	/	2.89	57.35	39.76	/
OC25 (20:1)	4	23.46 ± 0.43^{d}	13.93 ± 0.08^{d}	52.19 ± 1.53^{a}	10.42 ± 0.41^{b}	/	/	62.61	37.39	/
OC25 (20:1)	6	17.01 ± 0.16^{c}	11.76 ± 1.3^{c}	51.58 ± 0.86^{a}	18.6 ± 0.68^{c}	0.35 ± 0.00^{a}	0.7	70.53	28.77	53.14
OC25 (20:1)	8	15.13 ± 0.07^{b}	7.24 ± 1.1^{b}	50.6 ± 1.62^{a}	24.93 ± 0.27^{d}	0.76 ± 0.17^{b}	1.34	76.29	22.37	32.80
OC25 (20:1)	10	$13.66 \pm 0.5^{a*}$	$5.7 \pm 0.4^{a*}$	$50.45 \pm 0.48^{a*}$	$27.42 \pm 0.36^{e*}$	$0.99 \pm 0.1^{c*}$	3.78	78.86	19.36	26.69
OC50 (35:1)	2	24.31 ± 0.37^{e}	17.4 ± 1.27^{e}	50.17 ± 1.76^{c}	6.48 ± 0.33^{a}	/	2.64	58.29	41.71	/
OC50 (35:1)	4	21.31 ± 0.12^{d}	13.34 ± 0.89^{d}	49.07 ± 1.47^{c}	12.24 ± 1.2^{b}	2.78 ± 0.04^{a}	1.26	64.09	34.65	4.50
OC50 (35:1)	6	16.50 ± 0.18^{c}	8.17 ± 0.55^{c}	47.20 ± 1.21^{b}	22.11 ± 1.09^{c}	3.78 ± 0.16^{b}	1.84	73.49	24.67	5.96
OC50 (35:1)	8	15.97 ± 0.00^{b}	7.04 ± 0.67^{b}	42.63 ± 2.01^{a}	27.63 ± 0.88^{d}	4.53 ± 0.18^{c}	2.20	74.79	23.01	6.10
OC50 (35:1)	10	$12.04 \pm 0.70^{a**}$	$8.13 \pm 1.41^{a**}$	$40.55 \pm 1.19^{a**}$	$33.67 \pm 1.71^{e**}$	$4.44 \pm 0.78^{c**}$	3.17	79.66	18.17	6.19
OC75 (72:1)	2	19.14 ± 0.78^{e}	14.52 ± 0.12^{e}	62.12 ± 2.23^{b}	2.23 ± 0.03^{a}	0.48 ± 0.01^{a}	1.61	64.83	33.56	4.65
OC75 (72:1)	4	16.96 ± 0.67^{d}	11.44 ± 0.36^{d}	60.66 ± 1.15^{b}	5.72 ± 0.1^{b}	1.82 ± 0.07^{b}	3.40	68.2	28.4	3.14
OC75 (72:1)	6	14.45 ± 0.98^{c}	7.43 ± 0.87^{c}	57.34 ± 1.16^{a}	12.79 ± 0.74^{c}	3.01 ± 0.25^{c}	4.98	73.14	21.88	4.25
OC75 (72:1)	8	13.02 ± 0.43^{b}	6.35 ± 0.52^{b}	55.01 ± 1.7^{a}	20.13 ± 1.24^{d}	3.53 ± 0.66^{d}	1.96	78.67	19.37	5.70
OC75 (72:1)	10	$11.97 \pm 0.52^{a**}$	$6.15 \pm 0.94^{a**}$	$54.1 \pm 1.32^{a***}$	$22.06 \pm 2.02^{e**}$	$3.24 \pm 0.89^{d**}$	2.48	79.4	18.12	6.81

Different letters in the same column indicate significant difference. Different starts in the same column indicate significant difference (n = 3) ($p < 0.05$)

fatty acids were palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3). These findings align with those reported in the literature for *Rhodotorula* strains cultivated in synthetic or waste-derived effluent-based media (Shneider et al. 2013; Kot et al. 2017; Ribeiro et al. 2019; Keskin et al. 2023).

Moreover, results revealed that oleic acid, followed by linoleic acid and palmitic acid, were the most abundant fatty acids in the studied strain, particularly in OC50 medium (Table 3). Ratledge (1994) reported similar trends, highlighting oleic acid (C18:1) as the principal fatty acid (up to 70% w/w) accumulated by yeast cells, followed by linoleic acid (C18:2) (15–25% w/w) and palmitic acid (C16:0).

Furthermore, significant variations and shifts in the fatty acid profile of *R. glutinis* were observed across all media during the 10-day cultivation period. Indeed, palmitic acid and stearic acid exhibited a significant decrease, with the most significant decrease observed in OC50 (64.7%, 50.5%, and 21.7%, respectively, $p > 0.05$). Globally, oleic acid maintained relatively high proportions in all media (between 40–55%). Conversely, the levels of polyunsaturated fatty acids (PUFAs) increased significantly from day 2 to day 10 across all media ($p < 0.05$).

This trend was particularly pronounced in OC50 compared to OC25 and OC75, where *R. glutinis* biomass exhibited the highest linoleic and linolenic acid contents, increasing from 6.46% to 33.67% and from 0% to 4.44%, respectively (Table 6).

The simultaneous decrease in SFAs and increase in PUFAs is likely due to enhanced desaturase activity, a physiological adaptation maintaining membrane fluidity under stress such as nutrient limitation (Uemura 2012; Sakamoto & Murata 2002; Zhou et al. 2021). A high C/N ratio may further shift metabolic flux toward PUFA biosynthesis by increasing desaturation substrates and cofactors (Bellou et al. 2016).

Studies on *R. glutinis* confirm these trends: under higher C/N ratios or nutrient depletion, SFAs decrease and UFAs increase, with oleic acid dominant (Zhang et al. 2011; Shneider et al. 2013; Braunwald et al. 2013; Mast et al. 2014; Kot et al. 2020; Lopes et al. 2020; Keskin et al. 2023; Gientka et al. 2022). For example, Keskin et al. (2023) reported 63.94% oleic acid in undiluted OMWW medium at 30 °C, pH 6.0 after 6 days. Similarly, in this study oleic acid reached 57.34% in OC75 at day 6. Braunwald et al. (2013) found that at C/N 20 oleic acid was 39.9–44.4% and linoleic 31.2–42.3%, while palmitic and stearic acids were minimized; Zhang et al. (2011) noted linoleic acid rising from 8.5% to 12.7% over 9 days.

The composition of UFAs, MUFAs and PUFAs determines lipid applications: high MUFA/PUFA fractions are valued in food and feed, while high SFA fractions suit biodiesel (Arous et al. 2016; Nunes et al. 2024). In this study, lipids from early cultivation (2–4 days) were SFA-rich and biodiesel-oriented, whereas later stages showed increased PUFAs, especially in OC50 at day 10, indicating suitability for feed applications.

Moreover, the C18:2/C18:3 ratio observed in the OC50 and OC75 media, ranging from 4:1 to 6:1 **(**Table 6**)**, could be considered promising for animal nutrition, supporting

immune function, reducing inflammatory risks, and enhancing metabolic efficiency in livestock (Alagawany et al. 2019; Palupi et al. 2025). Additionally, the high content of oleic acid (C18:1) in all biomasses increases the digestibility and energy density of the lipids, which is particularly beneficial for improving feed conversion efficiency and supporting growth performance in monogastric animals such as poultry and swine (Piccinin et al. 2019; Coniglio et al. 2023). These findings indicate that microbial oils produced under OC50 and OC75 conditions could serve as viable and functional lipid sources in modern animal feeding strategies.

Total carotenoids content

The total carotenoids content in *R. glutinis* biomass cultivated on OC25, OC50, and OC75 media (pH 5.7), under 150 rpm agitation at 30 °C over a 10-day period, is displayed in Fig. 5C. In the OC25 medium (C/N 20:1), the total carotenoids content increased significantly from 256.01 ± 19 to 992.13 ± 38 µg g^{-1} (DW) between day 2 and day 6 ($p < 0.05$). After day 6 of fermentation, the total carotenoids content decreased sharply reaching a value of 277.51 ± 79 µg g^{-1} (DW) at day 10 ($p < 0.05$). In the OC50 medium (C/N 35:1), the same trend was observed. The total carotenoids content significantly reached the maximum content at day 8 with a value of 1281.8 ± 75 µg g^{-1} (DW) then decreased to reach a content of 755.0 ± 33 µg g^{-1} (DW) ($p < 0.05$). The maximum total carotenoid content detected in OC25 on day 6 and in OC50 on day 8, corresponding to the stationary phase, is attributed to nutrient depletion, which induces physiological stress and enhances carotenoid biosynthesis as a survival response (Byrtusová et al. 2021; Fakankun et al. 2021; Sereti et al. 2023). Furthermore, the decline in total carotenoids observed in OC25 and OC50 after days 6 and 8 can be attributed to severe depletion of nitrogen and other carotenoid precursors, which drastically increases the C/N ratio and may trigger *Rhodotorula* cells to shift from carotenoid synthesis toward lipid accumulation (Somashekar and Joseph 2000; Mussagy et al. 2020). Additionally, the nutrient-depleted medium may activate oxidative enzymes, increase oxidative stress and lead to the consumption of carotenoids, which act as antioxidants to support cell maintenance and stress resistance (Mantzouridou et al. 2002). Accordingly, in their study, Yimyoo et al. (2011) reported that *R. paludigenum* DMKU3-LPK4 produced the highest total carotenoid levels at a C/N ratio between 40 and 60, while lower (C/N 20) or higher (C/N 100) ratios led to a significant decrease in total carotenoid production. The stressful effect of a high C/N ratio on carotenoid production can be confirmed by the trend clearly observed in the OC75 medium (C/N 72:1) **(**Fig. 5C**)**, where total carotenoid content was significantly lower than those observed in OC25 and OC50, ranging from 157 ± 17 µg g^{-1} (DW) on day 2 to 409 ± 43 µg g^{-1} (DW) on day 10.

These findings indicate that both the initial C/N ratio and the fermentation duration are critical for effective carotenoid accumulation in *R. glutinis*. Moderate initial C/N ratios (around 20:1 to 35:1) combined with moderate to late stationary phase depending on the media (OC25 or OC50) seem to favor carotenoid biosynthesis.

Based on the biomass composition results, it is clearly shown that OC50 is the best medium, as it supports both high biomass production and lipid accumulation with a favorable profile, while also boosting carotenoid content. This demonstrates that OMWW and CW can be successfully used as an effective cultivation medium.

Total carotenoids extract (TCE) characterization

FTIR

Figure 6 represents the FTIR spectrum of TCE extracted from *R. glutinis* biomass cultivated on OC50 (pH 5.7) at 150 rpm under 30 °C over an 8-day period.

The TCE FTIR profile showed bands observed around 2800 cm^{-1} and 3500 cm^{-1}, with specific peaks at 2850.2 cm^{-1} and 3283.5 cm^{-1}, corresponding to the stretching vibrations of O–H groups, attributed to residual moisture or hydroxyl functional groups (Rubio-Diaz et al. 2010). The strong absorption peak at 1643.05 cm^{-1} is attributed to the C = O stretching vibration, indicative of conjugated ketones or ester groups commonly found in carotenoids (Quijano-Ortega et al. 2020). The region between 1000 cm^{-1} and 1500 cm^{-1} displays peaks at 1031.7 cm^{-1} and 1542.7 cm^{-1} corresponding to C-O and C = C stretching, as well as C-H bending vibrations, reflecting the presence of conjugated polyene chains (Schlücker et al. 2003; Berezin et al. 2005). The fingerprint region below 1000 cm^{-1} exhibits characteristic bands associated with the skeletal vibrations of the polyene backbone (Meléndez-Martínez et al. 2023), confirming the presence of carotenoid-specific structural motifs. Overall, this FTIR profile is consistent with the presence of highly conjugated double bonds, which are responsible for the optical and antioxidant properties of carotenoids.

The FTIR profile thus confirms the carotenoid nature of the extract. These results are similar to those reported by Mahajan et al. (2024) for carotenoids extracted from *Rhodotorula* sp. cultivated on potato waste (Mahajan et al. 2024).

Thermostability

The thermostability of TCE solution (~ 50 µg mL^{-1}) was evaluated by incubating the samples at varying temperatures (40, 60, 80, and 120 °C) over different time intervals (30, 60,

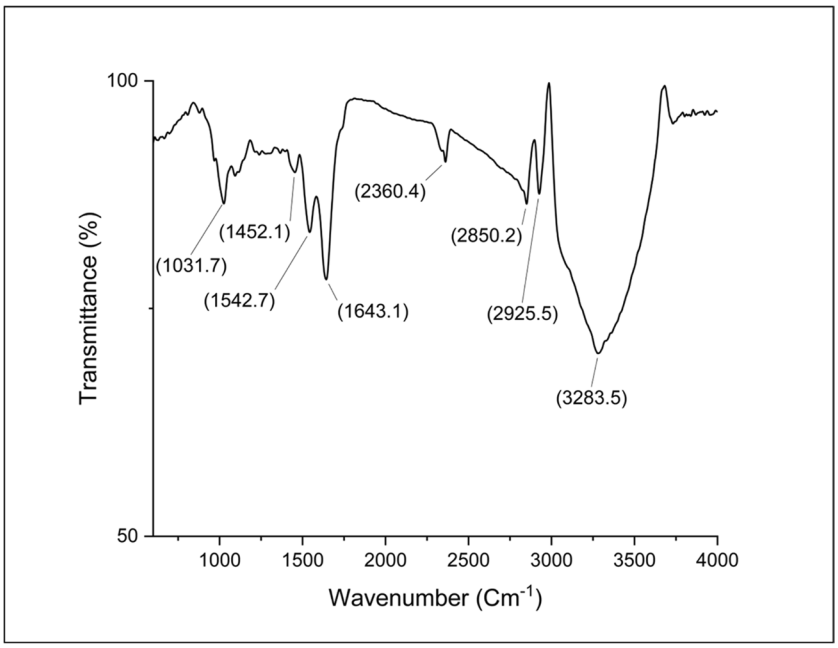


Fig. 6 FTIR spectrum of carotenoid produced by *R. glutinis* at OC50 (150 rpm, 30 °C, pH 5.7) over 8 days

120, 150, 180, 210, 240 and 270 min). As shown in Fig. 7, low heat treatment at 40 °C appears ideal for long-term preservation of TCE in food or feed matrices. TCE maintained over 85% of its initial stability (R_{TCE} = 86.57 ± 0.67%) even after 270 min at this temperature, indicating minimal thermal degradation. Moderate heating at 60 °C caused a significant decline in stability, with R_{TCE} values falling below 80% after 120 min and further declining to 68.17 ± 0.66% at 270 min ($p < 0.05$). These findings suggest that 60 °C may still be appropriate for short-duration applications such as low-temperature pasteurization, provided the exposure time remains under 60 min.

However, high temperatures of 80 °C and 120 °C induced rapid and extensive degradation, with R_{TCE} values dropping to 42.78 ± 1.22% and 27.54 ± 0.86%, respectively, after 270 min. Even after 30 min, visible degradation was noted at 80 °C. Therefore, such temperatures should be avoided during prolonged exposure in processing steps such as sterilization or cooking. Nevertheless, their use in rapid, short-duration processes may still be acceptable.

The degradation behavior observed in the present study is consistent with previous reports on carotenoid thermosensitivity, attributable to the structural vulnerability of conjugated polyene chains to thermal isomerization, oxidation,

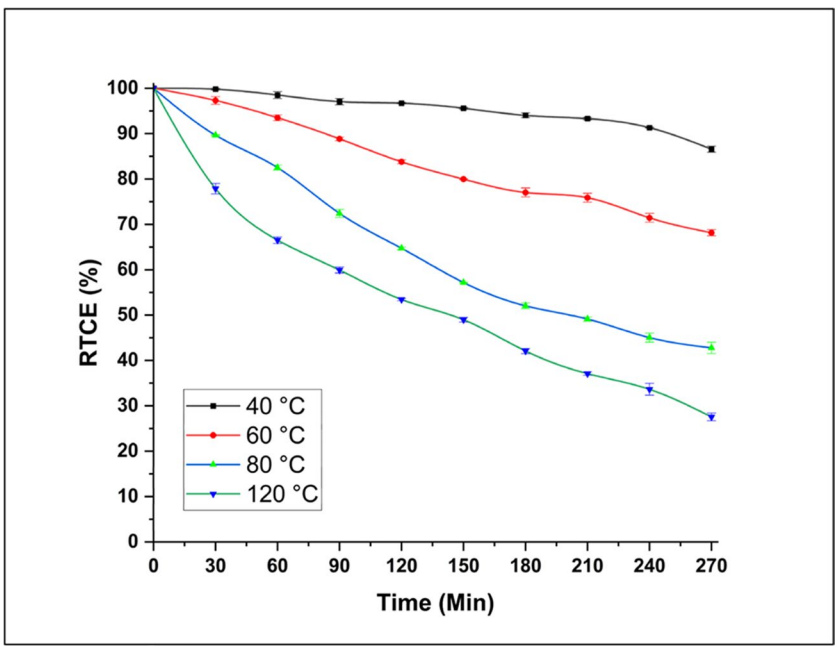


Fig. 7 Thermostability TCE from *R. glutinis* cultivated at OC50 at 40 °C, 60 °C, 80 °C and 120 °C. R_{TCE}: Relative TCE

and cleavage (Boon et al. 2010, Mordi et al. 2020; Yahia et al. 2017). This loss in structural integrity could be critical as it may affect the biofunctional and sensory qualities of TCE-rich products (Martin et al. 2019).

From a formulation perspective, these results underscore the need for appropriate thermal control during product development and storage. Moreover, this study supports the use of temperatures between 40 and 60 °C as a safe thermal processing window, particularly when combined with short exposure times. Future studies could focus on the kinetics of degradation, protective encapsulation techniques, or synergistic use of antioxidants to mitigate thermally induced breakdown and extend the usability of TCE under broader processing conditions.

To the best of our knowledge, only Mahajan and Nikhanj (2024) have examined thermal degradation in *Rhodotorula*-derived carotenoids, reporting similar trends with high stability up to 70 °C and notable browning beyond this point. Other studies involving plant, yeast, or microalgae-derived carotenoids also support these findings, reporting rapid fading or functional loss when exposed to temperatures ranging between 70 and 120 °C for 30 to 60 min (Rodriguez-Amaya et al. 2021; Šeregelj et al. 2022).

Antioxidant activity

The antioxidant capacity of the TCE was evaluated using two i*n vitro* antioxidant mechanisms. Based on the data presented in Fig. 8. TCE exhibited a concentration-dependent antioxidant effect. The antioxidant activity increased significantly with the increase of TCE concentrations from 6.43 to 102.8 µg mL^{-1}. At 102.8 µg mL^{-11}, the TCE exhibited significant activity of 96.6 ± 1.41% and 2.83 ± 0.08 for DPPH radical scavenging and reducing power tests, respectively.

The IC_{50} and EC_{50} values were 25.01 µg mL^{-1} and 15.77 µg mL^{-1} for the DPPH and reducing power assays, respectively, indicating the strong antioxidant activity of TCE via proton and electron donation. Nevertheless, these values were lower than those of BHA (control at the same concentrations range).

The potent antioxidant activity of carotenoids is mainly attributed to their extended system of conjugated double bonds, which generates an electron-rich structure effective in scavenging free radicals (Stahl and Sies 2003; Mandelli et al. 2012). Additionally, their molecular architecture characterized by cyclic β-ionone rings facilitates multiple antioxidant mechanisms, including electron transfer and hydrogen atom donation (Sandhiya et al. 2022; Abubakar et al. 2025).

Indeed, torularhodin, comprising 13 double bonds, was identified as one of the most effective carotenoids in terms of antioxidant activity (Kot et al. 2018; Sereti et al. 2023, 2024). The present findings are consistent with those reported by Mahajan et al. (2024), Keceli et al. (2013), and Ribeiro et al. (2023), who also emphasized the strong antioxidant potential of carotenoids derived from *Rhodotorula* strains.

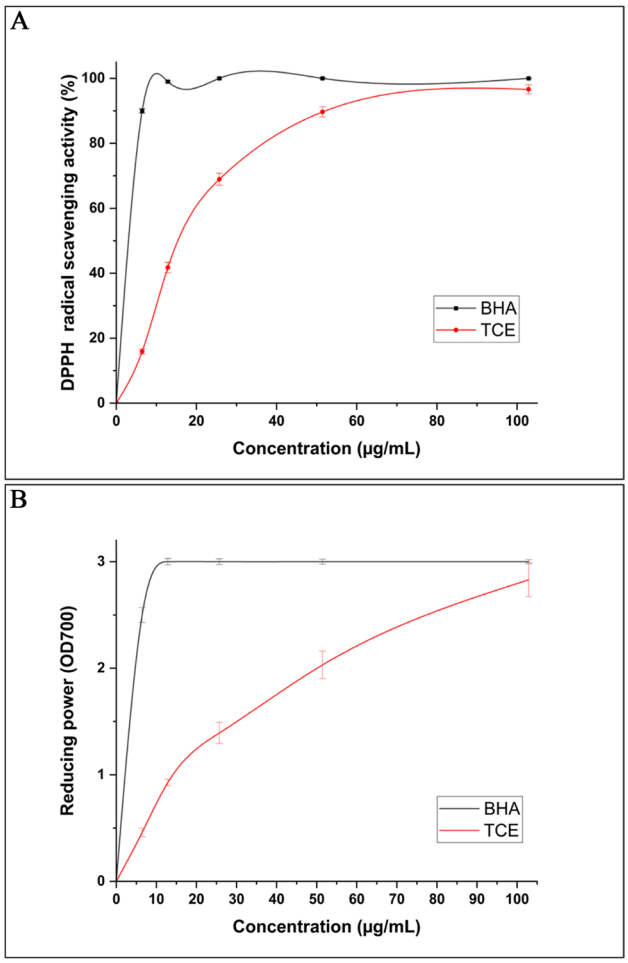


Fig. 8 DPPH radical scavenging activity (**A**) and reducing power (**B**) TCE extracted *R. glutinis* cultivated in OC50 (150 rpm, 30 °C, pH 5.7, 8 days)

Antibacterial properties

Table 7 summarized the average inhibition values for the antibacterial properties of TCE. The use of TCE at 50 µg mL^{-1} showed inhibition zone diameters of 13.0, 10.0,

Table 7 Antibacterial activity of TCE from *R. glutinis* biomass cultivated in OC50

	Inhibition zone (mm)	
	TCE (50 µg mL^{-1})	Gentamicin
E. coli (ATCC 25922)	13.00 ± 0.41[a]	30.00[b]
B. Subtilis (ATCC 15442)	11.50 ± 0.71[a]	25.00[b]
S. aureus (ATCC 25923)	15.50 ± 0.71[a]	34.00[b]
P. aerogenosa (ATCC 6633)	0.00 ± 0.00[a]	0.00[a]

Different letters in the same line for the same cultivation medium indicate significant difference ($p<0.05$) (n=3)

15.5, and 0.0 mm against *E. coli*, *B. subtilis*, *S. aureus*, and *P. aeruginosa*, respectively **(SI.2)**. Nevertheless, the antibacterial capacity of TCE was significantly lower than that of gentamicin (used as control) with inhibition zone diameters higher than 25 mm ($p < 0.05$). The activity against *E. coli* and *S. aureus* supports the TCE relevance for food preservation. Inhibition of *B. subtilis*, a non-pathogenic Gram-positive bacterium, indicates that potential effects on beneficial strains should be considered when TCE is used as a coloring or preservative agent in food or feed formulation.

The antibacterial activity of TCE could be attributed to the presence of torularhodin as the major carotenoids in *R. glutinis* (Zoz et al. 2015; Kot et al. 2018; Sebastian et al. 2023). Several studies confirmed the antibacterial properties of carotenoids extracted from red yeasts, including various *Rhodotorula* strains, which have demonstrated significant inhibitory effects against both Gram-positive and Gram-negative bacteria (Karanjgaokar and Tarfe 2017; Serati et al. 2024; Mahajan et al. 2024).

Conclusion

OMWW is a major agro-industrial effluent characterized by high organic and phenolic content, making its valorization a continuing challenge. In this study, *R. glutinis* was investigated as a biotechnological tool to mitigate this issue. To enable microbial growth in OMWW possible, CW was incorporated as a dual-functional agent, serving both as a nitrogen source and a diluting medium, thereby providing a more suitable C/N ratio for microbial development. Under these conditions, when cultivated in OMWW-CW mixtures (25/75, 50/50, 75/25, 100/0, v/v), *R. glutinis* achieved the highest biomass concentration (>5 g L^{-1}) after 3 days in the 25% OMWW and 75% CW mixture, followed by the 50% OMWW and 50% CW mixture after 5 days. However, lipid and carotenoid accumulation peaked when *R. glutinis* was cultivated in a 50% OMWW and 50% CW medium for 8 days. Moreover, this study demonstrated that *R. glutinis* effectively depolluted OMWW-CW mixtures, especially when CW was added at 50% or 75% (v/v), by reducing COD and nearly eliminating hydroxytyrosol and tyrosol. The 50% OMWW and 50% CW mixture was identified as the most favorable for lipid and carotenoid production while simultaneously enhancing wastewater bioremediation. Furthermore, carotenoids extracted from *R. glutinis* cultivated in the 50% OMWW and 50% CW mixture retained 50% stability at 70 °C after 50 min of exposure and exhibited strong antioxidant and antibacterial activities, suggesting their potential application as bioactive ingredients in food formulations. Overall, these findings highlight a promising biotechnological approach in which combining OMWW and CW provides a feasible valorization route that not only supports the microbial production of high-value compounds but also couples effluent remediation with environmental sustainability. The present study also establishes the basis for subsequent scale-up efforts aimed at advancing the process toward pilot- and industrial-level applications.

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Author contribution IC: Funding acquisition, Project administration, Conceptualization, Resources, Methodology, Investigation, Data curation, Validation, Writing-original draft, Writing-review & editing. AMK and YBM: Writing-original draft, Writing-review & editing. SD, AHB, ES, FA, FAV, AB and CA: Contributed to the investigation.

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Data availability Data will be made available on request.

Declarations

Competing interest The authors declare no competing interests.

References

Abeln F, Chuck CJ (2021) The history, state of the art and future prospects for oleaginous yeast research. Microb Cell Fact 20:221. https://doi.org/10.1186/s12934-021-01712-1

Abubakar H, Astuti RI, Batubara I et al (2025) Investigating antioxidant activity of carotenoid compound from *Paracoccus haeundaensis* SAB E11 at the cellular level in *Schizosaccharomyces pombe* ARC039 yeast model. J Appl Pharm Sci 15(3):183–193. https://doi.org/10.7324/JAPS.2025.204168

Achak M, Boumya W, Ouazzani N, Mandi L (2019) Preliminary evaluation of constructed wetlands for nutrients removal from olive mill wastewater (OMW) after passing through a sand filter. Ecol Eng 136:141–151. https://doi.org/10.1016/j.ecoleng.2019.06.007

AFNOR (1984) Recueil de normes françaises. Produits agricoles alimentaires : Directives générales pour le dosage de l'azote avec minéralisation selon la méthode de Kjeldahl. AFNOR, Paris

Ahmad T, Aadil RM, Ahmed H (2019) Treatment and utilization of dairy industrial waste: a review. Trends Food Sci Technol 88:361–372. https://doi.org/10.1016/j.tifs.2019.04.003

Akkam Y, Zaitoun M, Aljarrah I, Jaradat A, Hmedat A, Alhmoud H, Rababah T, Almajwal A, Al-Rayyan N (2024) Effective detoxification of olive mill wastewater using multi-step surfactant-based treatment: assessment of environmental and health impact. Molecules 29(18):4284. https://doi.org/10.3390/molecules29184284

Alagawany M, Elnesr SS, Farag MR, Abd El-Hack ME, Khafaga AF, Taha AE, Tiwari R, Yatoo MI, Bhatt P, Khurana SK, Dhama K (2019) Omega-3 and omega-6 fatty acids in poultry nutrition: effect on production performance and health. Animals 9:573. https://doi.org/10.3390/ani9080573

Al-Bsoul A, Al-Shannag M, Tawalbeh M, Al-Taani AA, Lafi WK, Al-Othman A, Alsheyab M (2020) Optimal conditions for olive mill wastewater treatment using ultrasound and advanced oxidation processes. Sci Total Environ 700:134576. https://doi.org/10.1016/j.scitotenv.2019.134576

Allahkarami S, Sepahi AA, Hosseini H, Razavi MR (2021) Isolation and identification of carotenoid-producing *Rhodotorula* sp. from Pinaceae forest ecosystems and optimization of *in vitro* carotenoid production. Biotechnol Rep 32:e00687. https://doi.org/10.1016/j.btre.2021.e00687

Alrowais R, Yousef RS, Ahmed OK (2023) Enhanced detoxification methods for the safe reuse of treated olive mill wastewater in irrigation. Environ Sci Eur 35:95. https://doi.org/10.1186/s12302-023-00797-2

Ammari M, Zerrouk M, Zoufri I, El-Byari Y, Mazrha A, Mrizak FE, Merzouki M (2025) Removal of organic compounds from olive mill wastewater using an eco-friendly adsorbent: characterization, kinetics, isotherms, thermodynamics, and interaction analysis. Sci Afr 27:e02612. https://doi.org/10.1016/j.sciaf.2025.e02612

AOAC (2000) Official methods of analysis. 17th edn. The Association of Official Analytical Chemists, Gaithersburg, MD, USA. Methods 925.10, 65.17, 974.24, 992.16.

Aouidi F, Gannoun H, Ben Othman N (2009) Improvement of fermentative decolorization of olive mill wastewater by *Lactobacillus paracasei* by cheese whey's addition. Process Biochem 44(5):597–601. https://doi.org/10.1016/j.procbio.2009.02.014

APHA (1998) Standard methods for the examination of water and wastewater. 20th edn. American Public Health Association, American Water Works Association, and Water Environmental Federation, Washington DC

Arous F, Azabou S, Jaouani A (2016) Biosynthesis of single-cell biomass from olive mill wastewater by newly isolated yeasts. Environ Sci Pollut Res Int 23(7):6783–6792. https://doi.org/10.1007/s11356-015-5924-2

Arous F, Hamdi C, Kmiha S, Khammassi N, Ayari A, Neifar M, Mechichi T, Jaouani A (2018) Treatment of olive mill wastewater through employing sequencing batch reactor: performance and microbial diversity assessment. 3 Biotech. https://doi.org/10.1007/s13205-018-1486-6

Bellou S, Triantaphyllidou IE, Mizerakis P, Aggelis G (2016) High lipid accumulation in *Yarrowia lipolytica* cultivated under double limitation of nitrogen and magnesium. J Biotechnol 234:116–126. https://doi.org/10.1016/j.jbiotec.2016.08.001

Ben Sassi A, Boularbah A, Jaouad A, Walker G, Boussaid A (2006) A comparison of olive oil mill wastewaters (OMW) from three different processes in Morocco. Process Biochem 41(1):74–78. https://doi.org/10.1016/j.procbio.2005.03.074

Beopoulos A, Cescut J, Haddouche R, Uribelarrea JL, Molina-Jouve C, Nicaud JM (2009) *Yarrowia lipolytica* as a model for bio-oil production. Prog Lipid Res 48:375–387. https://doi.org/10.1016/j.plipres.2009.08.005

Berezin KV, Nechaev VV (2005) Calculation of the IR spectrum and the molecular structure of β-carotene. J Appl Spectrosc 72:164–171. https://doi.org/10.1007/s10812-005-0049-x

Bersuder P, Hole M, Smith G (1998) Antioxidants from a heated histidine-glucose model system. I: Investigation of the antioxidant role of histidine and isolation of antioxidants by high-performance liquid chromatography. J Am Oil Chem Soc 75:181–187. https://doi.org/10.1007/s11746-998-0030-y

Boon CS, McClements DJ, Weiss J, Decker EA (2010) Factors influencing the chemical stability of carotenoids in foods. Crit Rev Food Sci Nutr 50:515–532. https://doi.org/10.1080/10408390802565889

Bougherara S, Boucetta N, Lecheb F, Medjdoub F, Delleci K, Belkhir M (2021) Characterization and valorisation of olive by-products from a traditional oil mill in Béjaia. Algerian J Environ Sci Technol 7(1):1819–1824

Boutafda A, Zegzouti Y, El Fels L, Ouhdouch Y, Lebrihi A, Bekkaoui F, Hafidi M (2019) Olive mill wastewater biological assessment: optimization and identification of compounds degrading yeasts. Desalin Water Treat 149:129–137. https://doi.org/10.5004/dwt.2019.23869

Braunwald T, Schwemmlein L, Graeff-Hönninger S, French WT, Hernandez R, Holmes WE, Claupein W (2013) Effect of different C/N ratios on carotenoid and lipid production by *Rhodotorula glutinis*. Appl Microbiol Biotechnol 97:6581–6588. https://doi.org/10.1007/s00253-013-5005-8

Broach JR (2012) Nutritional control of growth and development in yeast. Genetics 192:73–105. https://doi.org/10.1534/genetics.111.135731

Byrtusová D, Szotkowski M, Kurowska K, Shapaval V, Márová I (2021) *Rhodotorula kratochvilovae* CCY 20–2-26 the source of multifunctional metabolites. Microorganisms 9:1280. https://doi.org/10.3390/microorganisms9061280

Cadete RM, Lopes MR, Rosa CA (2017) Yeasts associated with decomposing plant material and rotting wood. In: Buzzini P, Lachance MA, Yurkov A (eds) Yeasts in Natural Ecosystems: Diversity. Springer, Cham. https://doi.org/10.1007/978-3-319-62683-3_9

Cai Y, Zhai L, Fang X, Wu K, Liu Y, Cui X, Wang Y, Yu Z, Ruan R, Liu T, Zhang Q (2022) Effects of C/N ratio on the growth and protein accumulation of heterotrophic *Chlorella* in broken rice hydrolysate. Biotechnol Biofuels 15:102. https://doi.org/10.1186/s13068-022-02204-z

Caporusso A, De Bari I, Valerio V, Albergo R, Liuzzi F (2021) Conversion of cardoon crop residues into single cell oils by *Lipomyces tetrasporus* and *Cutaneotrichosporon curvatus*: process optimizations to overcome the microbial inhibition of lignocellulosic hydrolysates. Ind Crops Prod 159:113030. https://doi.org/10.1016/j.indcrop.2020.113030

Chebbi H, Leiva-Candia D, Carmona-Cabello M, Jaouani A, Pilar Dorado M (2019) Biodiesel production from microbial oil provided by oleaginous yeasts from Olive oil mill wastewater growing on industrial glycerol. Ind Crops Prod 139:111535. https://doi.org/10.1016/j.indcrop.2019.111535

Cheng YT, Yang CF (2016) Using strain *Rhodotorula mucilaginosa* to produce carotenoids using food wastes. J Taiwan Inst Chem Eng 61:270–275. https://doi.org/10.1016/j.jtice.2015.12.027

Chiavola A, Farabegoli G, Antonetti F (2014) Biological treatment of olive mill wastewater in a sequencing batch reactor. Biochem Eng J 85:71–78. https://doi.org/10.1016/j.bej.2014.02.004

Chikhi K, Bendi Djelloul MCE (2022) The olive oil market in the Mediterranean: what are marketing strategies for Algeria? Contemporary Agriculture 71(1–2):28–37. https://doi.org/10.2478/contagri-2022-0005

Cho DH, Chae HJ, Kim EY (2001) Synthesis and characterization of a novel extracellular polysaccharide by *Rhodotorula glutinis*. Appl

Biochem Biotech 95(3):183–194. https://doi.org/10.1385/abab:95:3:183

Christwardana M, Khoirunnisa K, Asy'ari M, Hadiyanto H (2025) Evaluating nitrogen sources for enhanced halophilic bacteria growth, electron transfer, and microbial fuel cell performance. Chemosphere 378:144397. https://doi.org/10.1016/j.chemosphere.2025.144397

Coniglio S, Shumskaya M, Vassiliou E (2023) Unsaturated fatty acids and their immunomodulatory properties. Biology 12(2):279. https://doi.org/10.3390/biology12020279

Costa GS, Martinez-Burgos WJ, dos Reis GA, Puche YP, Vega FR, Rodrigues C, Serra JL, de Campos SM, Soccol CR (2024) Advances in biomass and microbial lipids production: trends and prospects. Processes 12:2903. https://doi.org/10.3390/pr12122903

Crialesi A, Mazzarotta B, Santalucia M, Di Caprio F, Pozio A, Santucci A, Farina L (2022) Exploiting olive mill wastewater via thermal conversion of the organic matter into gaseous biofuel – a case study. Energies 15:2901. https://doi.org/10.3390/en15082901

Cuffaro D, Bertolini A, Bertini S, Ricci C, Cascone MG, Danti S, Saba A, Macchia M, Digiacomo M (2023) Olive mill wastewater as source of polyphenols with nutraceutical properties. Nutrients 15(17):3746. https://doi.org/10.3390/nu15173746

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28(3):350–356. https://doi.org/10.1021/ac60111a017

Dulak K, Sordon S, Matera A, Kozak B, Huszcza E, Popłoński J (2022) Novel flavonoid C8 hydroxylase from *Rhodotorula glutinis*: identification, characterization, and substrate scope. Microb Cell Fact 21(1):175. https://doi.org/10.1186/s12934-022-01899-x

El Yamani M, Sakar EH, Boussakouran A, Ghabbour N, Rharrabti Y (2019) Physicochemical and microbiological characterization of olive mill wastewater (OMW) from different regions of northern Morocco. Environ Technol 41(23):3081–3093. https://doi.org/10.1080/09593330.2019.1597926

El-Abbassi A, Kiai H, Hafidi A (2012) Phenolic profile and antioxidant activities of olive mill wastewater. Food Chem 132(1):406–412. https://doi.org/10.1016/j.foodchem.2011.11.013

El-Bialy HAA, Gomaa OM, Azab KS (2011) Conversion of oil waste to valuable fatty acids using oleaginous yeast. World J Microbiol Biotechnol 27:2791–2798. https://doi.org/10.1007/s11274-011-0755-x

Elfeky N, Elmahmoudy M, Zhang Y, Guo J, Bao Y (2019) Lipid and carotenoid production by *Rhodotorula glutinis* with a combined cultivation mode of nitrogen, sulfur, and aluminium stress. Appl Sci 9(12):2444. https://doi.org/10.3390/app9122444

El-Gohary FA, Badawy MI, El-Khateeb MA, El-Kalliny AS (2009) Integrated treatment of olive mill wastewater (OMW) by the combination of Fenton's reaction and anaerobic treatment. J Hazard Mater 162(2–3):1536–1541. https://doi.org/10.1016/j.jhazmat.2008.06.098

Elkacmi, R., Bennajah, M. (2018). New Techniques for Treatment and Recovery of Valuable Products from Olive Mill Wastewater. In Handbook of Environmental Materials Management (pp. 1–20). Springer International Publishing. https://doi.org/10.1007/978-3-319-58538-3_157-1

Elkacmi, R., Bennajah, M. (2019). New Techniques for Treatment and Recovery of Valuable Products from Olive Mill Wastewater. In Handbook of Environmental Materials Management (pp. 1839–1858). Springer International Publishing. https://doi.org/10.1007/978-3-319-73645-7_157

Enaime G, Dababat S, Wichern M, Lübken M (2024) Olive mill wastes: from wastes to resources. Environ Sci Pollut Res 31(14):20853–20880. https://doi.org/10.1007/s11356-024-32468-x

Esteves BM, Fernandes R, Morales-Torres S, Figueiredo JL, Silva AMT (2022) Integration of catalytic wet peroxidation and membrane distillation processes for olive mill wastewater treatment and water recovery. Chem Eng J 448:137586. https://doi.org/10.1016/j.cej.2022.137586

Fakankun I, Fristensky B, Levin DB (2021) Genome sequence analysis of the oleaginous yeast, *Rhodotorula diobovata*, and comparison of the carotenogenic and oleaginous pathway genes and gene products with other oleaginous yeasts. J Fungi 7:320. https://doi.org/10.3390/jof7040320

Fakas S, Galiotou-Panayotou M, Papanikolaou S, Komaitis M, Aggelis G (2007) Compositional shifts in lipid fractions during lipid turnover in *Cunninghamella echinulata*. Enzyme Microb Technol 40(5):1321–1327. https://doi.org/10.1016/j.enzmictec.2006.10.005

Fakharedine N, Ouadghiri M, Amar M, Winterton P, Hafidi M, Ouhdouch Y (2011) Isolation and identification of a yeast strain involved in the degradation of Marrakech olive mill wastewater. Eur Asian J Biosci 137:127–137

Fernandes MJ, Gomes J, Carvalho P, Martins RC, Domingues E (2025) Phenolic compounds recovery to treat and valorize olive mill wastewater: technologies overview. Chem Eng Sci 305:121145. https://doi.org/10.1016/j.ces.2024.121145

Filippousi R, Diamantopoulou P, Stavropoulou M, Makris DP, Papanikolaou S (2022) Lipid production by *Rhodosporidium toruloides* from biodiesel-derived glycerol in shake flasks and bioreactor: impact of initial C/N molar ratio and added onion-peel extract. Process Biochem 123:52–62. https://doi.org/10.1016/j.procbio.2022.10.008

Fiorentino A, Gentili A, Isidori M, Monaco P, Nardelli A, Parrella A, Temussi F (2003) Environmental effects caused by olive mill wastewaters: toxicity comparison of low-molecular-weight phenol components. J Agric Food Chem 51(4):1005–1009. https://doi.org/10.1021/jf020887d

Fleyfel LM, Karpel Vel Leitner N, Deborde M, Matta J, El Najjar NH (2022) Olive oil liquid wastes–Characteristics and treatments: a literature review. Process Saf Environ Prot 168:1031–1048. https://doi.org/10.1016/j.psep.2022.10.035

Fleyfel LM, Matta J, Sayegh NF, El Najjar NH (2024) Olive mill wastewater treatment using coagulation/flocculation and filtration processes. Heliyon 10(22):e40348. https://doi.org/10.1016/j.heliyon.2024.e40348

Foti P, Romeo FV, Russo N, Pino A, Vaccalluzzo A, Caggia C, Randazzo CL (2021) Olive mill wastewater as renewable raw materials to generate high added-value ingredients for agro-food industries. Appl Sci 11:7511. https://doi.org/10.3390/app11675 11

Garrido-Baserba M, Sedlak DL, Molinos-Senante M, Barnosell I, Schraa O, Rosso D, Verdaguer M, Poch M (2024) Using water and wastewater decentralization to enhance the resilience and sustainability of cities. Nat Water 2:953–974. https://doi.org/10.1038/s44221-024-00303-9

Ghilardi C, Sanmartin NP, Carelli AA, Borroni V (2020) Evaluation of olive mill waste as substrate for carotenoid production by *Rhodotorula mucilaginosa*. Bioresour Bioprocess 7:52. https://doi.org/10.1186/s40643-020-00341-7

Ghilardi C, Sanmartin NP, Rodríguez Gutiérrez G, Monetta P, Arroyo-López FN, Hornero-Méndez D, Carelli AA, Borroni V (2022) Influence of olive mill waste phenolic compounds levels on carotenoid production by *Rhodotorula* spp. Process Biochem 120:275–286. https://doi.org/10.1016/j.procbio.2022.06.013

Gientka I, Wirkowska-Wojdyła M, Ostrowska-Ligęza E, Janowicz M, Reczek L, Synowiec A, Błażejak S (2022) Enhancing red yeast biomass yield and lipid biosynthesis by using waste nitrogen source by glucose fed-batch at low temperature. Microorganisms 10:1253. https://doi.org/10.3390/microorganisms10061253

Girometti E, Frascari D, Pinelli D, Di Federico V, Libero G, Ciriello V (2025) Polyphenol adsorption and recovery from olive mill

wastewater: a model reduction-based optimization and economic assessment. J Environ Chem Eng 13(3):116370. https://doi.org/10.1016/j.jece.2025.116370

Gong G, Liu L, Zhang X, Tan T (2019) Comparative evaluation of different carbon sources supply on simultaneous production of lipid and carotene of *Rhodotorula glutinis* with irradiation and the assessment of key gene transcription. Bioresour Technol 288:121559. https://doi.org/10.1016/j.biortech.2019.121559

Gueboudji Z, Addad D, Kadi K, Nagaz K, Secrafi M, Ben Yahya L, Lachehib B, Abdelmalek A (2022) Biological activities and phenolic compounds of olive oil mill wastewater from Abani, endemic Algerian variety. Sci Rep 12:6042. https://doi.org/10.1038/s41598-022-10052-y

Gueboudji Z, Kadi K, Nagaz K (2022) Phytochemical screening and assessment of the antioxidant activity of bio-phenols of olive oil mill wastewater from the cold extraction of olive oil in Khenchela region. Eastern Algeria. J Ren Energies 1(1):1044. https://doi.org/10.54966/jreen.v1i1.1044

Hafidi A, Pioch D, Ajana H (2005) Effects of a membrane-based soft purification process on olive oil quality. Food Chem 92(4):607–613. https://doi.org/10.1016/j.foodchem.2004.08.046

Hong-Wei Y, Chun-Yu H, Wei-Siang L (2019) A cost-efficient way to obtain lipid accumulation in the oleaginous yeast *Rhodotorula glutinis* using supplemental waste cooking oils (WCO). J Taiwan Inst Chem Eng 97:523–530. https://doi.org/10.1016/j.jtice.2019.02.012

Issa A, El Riachy M, Bou-Mitri C, Doumit J, Skaff W, Karam L (2023) Influence of geographical origin, harvesting time and processing system on the characteristics of olive-mill wastewater: a step toward reducing the environmental impact of the olive oil sector. Environ Technol Innov 32:103365. https://doi.org/10.1016/j.eti.2023.103365

Jaradat AQ, Gharaibeh S, Abu Irjei M (2018) The application of solar distillation technique as a mean for olive mill wastewater management. Water Environ J 32:134–140. https://doi.org/10.1111/wej.12308

Jarboui R, Baati H, Fetoui F, Gargouri A, Gharsallah N, Ammar E (2012) Yeast performance in wastewater treatment: case study of *Rhodotorula mucilaginosa*. Environ Technol 33(7–9):951–960. https://doi.org/10.1080/09593330.2011.603753

Javan K, Altaee A, BaniHashemi S, Darestani M, Zhou J, Pignatta G (2024) A review of interconnected challenges in the water–energy–food nexus: urban pollution perspective towards sustainable development. Sci Total Environ 912:169319. https://doi.org/10.1016/j.scitotenv.2023.169319

Jiru TM, Groenewald M, Pohl C, Steyn L, Abate D, Krumov N (2017) Optimization of cultivation conditions for biotechnological production of lipid by Rhodotorula kratochvilovae (syn. Rhodosporidium kratochvilovae) SY89 for biodiesel preparation. 3 Biotech 7:145. https://doi.org/10.1007/s13205-017-0769-7

Kamalesh R, Saravanan A, Yaashikaa PR, Vickram AS, Thiruvengadam S (2025a) Recent insights into membrane separation technology for partitioning of nutritional components in dairy products - mechanism and applications. Int Dairy J 169:106340. https://doi.org/10.1016/j.idairyj.2025.106340

Kamalesh R, Saravanan A, Yaashikaa PR, Vijayasri K (2025b) Innovative approaches to harnessing natural pigments from food waste and by-products for eco-friendly food coloring. Food Chem 463:141519. https://doi.org/10.1016/j.foodchem.2024.141519

Kaniewski D, Marriner N, Terral JF, Besnard G, Tsitsou L, Topsakal J, Morhange C, Otto T, Luce F, Cheddadi R (2025) Olive production in the 21st century will be threatened by water stress and declining solar activity. Commun Earth Environ. https://doi.org/10.1038/s43247-025-02256-7

Karakaya A, Laleli Y, Takaç S (2012) Development of process conditions for biodegradation of raw olive mill wastewater by *Rhodotorula glutinis*. Int Biodeterior Biodegrad 75:75–82. https://doi.org/10.1016/j.ibiod.2012.09.005

Karakaya A, Bozkoyunlu G, Laleli Y, Takaç S (2014) Development of pH adjustment-based operational strategy to increase total phenol removal rate in biodegradation of olive mill wastewater by *Rhodotorula glutinis*. Desalin Water Treat 52(37–39):7277–7281. https://doi.org/10.1080/19443994.2013.823357

Karamerou EE, Webb C (2019) Cultivation modes for microbial oil production using oleaginous yeasts – a review. Biochem Eng J 151:107322. https://doi.org/10.1016/j.bej.2019.107322

Karanjgaokar DR, Tarf KS (2017) Isolation of pigmented yeasts, extraction of pigment and study of antimicrobial property of its pigment. Int J Curr Microbiol App Sci 6(7):664–672. https://doi.org/10.20546/ijcmas.2017.607.081

Katayama-Hirayama K, Tobita S, Hirayama K (1991) Degradation of phenol by yeast *Rhodotorula*. J Gen Appl Microbiol 37(2):147–156. https://doi.org/10.2323/jgam.37.147

Keceli TM, Erginkaya Z, Turkkan E, Kaya U (2013) Antioxidant and antibacterial effects of carotenoids extracted from *Rhodotorula-glutinis* strains. Asian J Chem 25:42–50. https://doi.org/10.14233/ajchem.2013.12377

Keskin A, Ünlü AE, Takaç S (2023) Utilization of olive mill wastewater for selective production of lipids and carotenoids by *Rhodotorula glutinis*. Appl Microbiol Biotechnol 107:4973–4985. https://doi.org/10.1007/s00253-023-12625-x

Khdair IA, Abu-Rumman G (2017) Evaluation of the environmental pollution from olive mills wastewater. Fresenius Environ Bull 26:2537–2540

Khelouf I, Jabri Karoui I, Lakoud A, Hammami M, Abderrabba M (2023) Comparative chemical composition and antioxidant activity of olive leaves *Olea europaea* L. of Tunisian and Algerian varieties. Heliyon 9:e22217. https://doi.org/10.1016/j.heliyon.2023.e22217

Konzock O, Zaghen S, Fu J, Kerkhoven EJ (2022) Urea is a drop-in nitrogen source alternative to ammonium sulphate in *Yarrowia lipolytica*. iScience 25(12):105703. https://doi.org/10.1016/j.isci.2022.105703

Kot AM, Błażejak S, Gientka I, Kieliszek M, Bryś J (2018) Torulene and torularhodin: "new" fungal carotenoids for industry? Microb Cell Fact 17:49. https://doi.org/10.1186/s12934-018-0893-z

Kot AM, Błażejak S, Kieliszek M, Gientka I, Piwowarek K, Brzezińska R (2020) Production of lipids and carotenoids by *Rhodotorula gracilis* ATCC 10788 yeast in a bioreactor using low-cost wastes. Biocatal Agric Biotechnol 26:101634. https://doi.org/10.1016/j.bcab.2020.101634

Kot AM, Błażejak S, Kurcz A, Bryś J, Gientka I, Bzducha-Wróbel A, Maliszewska M, Reczek L (2017) Effect of initial pH of medium with potato wastewater and glycerol on protein, lipid and carotenoid biosynthesis by *Rhodotorula glutinis*. Electron J Biotechnol 27:25–31. https://doi.org/10.1016/j.ejbt.2017.01.007

Kot AM, Błażejak S, Kurcz A et al (2016) *Rhodotorula glutinis*- potential source of lipids, carotenoids, and enzymes for use in industries. Appl Microbiol Biotechnol 100:6103–6117. https://doi.org/10.1007/s00253-016-7611-8

Kuznetsova MV, Nesterova LY, Mihailovskaya VS, Selivanova PA, Kochergina DA, Karipova MO, Valtsifer IV, Averkina AS, Starčič Erjavec M (2025) Nosocomial *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus*: sensitivity to chlorhexidine-based biocides and prevalence of efflux pump genes. Int J Mol Sci 26(1):355. https://doi.org/10.3390/ijms26010355

Ladhari A, Zarrelli A, Ghannem M, Ben Mimoun M (2021) Olive wastes as a high-potential by-product: variability of their phenolic profiles, antioxidant and phytotoxic properties. Waste Biomass Valor 12:3657–3669. https://doi.org/10.1007/s12649-020-01256-2

Lakshmidevi R, Ramakrishnan B, Ratha SK, Bhaskar S, Chinnasamy S (2021) Valorisation of molasses by oleaginous yeasts for single cell oil (SCO) and carotenoids production. Environ Technol Innov 21:101281. https://doi.org/10.1016/j.eti.2020.101281

Ledesma-Amaro R, Dulermo R, Niehus X, Nicaud JM (2016) Combining metabolic engineering and process optimization to improve production and secretion of fatty acids. Metab Eng 38:38–46. https://doi.org/10.1016/j.ymben.2016.06.004

Lei C, Guo X, Zhang M, Zhou X, Ding N, Ren J, Liu M, Jia C, Wang Y, Zhao J, Dong Z, Lu D (2024) Regulating the metabolic flux of pyruvate dehydrogenase bypass to enhance lipid production in *Saccharomyces cerevisiae*. Commun Biol 7:1399. https://doi.org/10.1038/s42003-024-07103-7

Lenzuni M, D'Agostino G, Perego P, Converti A, Casazza AA (2025) Insights into the effects of phenolic compounds on the growth of *Chlorella vulgaris*: the case of olive mill wastewater. Sci Total Environ 958:177944. https://doi.org/10.1016/j.scitotenv.2024.177944

Lim J, Zhang X, Ferruzzi MG, Hamaker BR (2019) Starch digested product analysis by HPAEC reveals structural specificity of flavonoids in the inhibition of mammalian α-amylase and α-glucosidases. Food Chem 288:413–421. https://doi.org/10.1016/j.foodchem.2019.02.117

Liu P, Wen S, Zhu S, Hu X, Wang Y (2025) Microbial degradation of soil organic pollutants: mechanisms, challenges, and advances in forest ecosystem management. Processes 13(3):916. https://doi.org/10.3390/pr13030916

Lopes HJS, Bonturi N, Kerkhoven EJ, Miranda EA, Lahtvee PJ (2020) C/N ratio and carbon source-dependent lipid production profiling in *Rhodotorula toruloides*. Appl Microbiol Biotechnol 104:2639–2649. https://doi.org/10.1007/s00253-020-10386-5

Machado WRC, Murari CS, Duarte ALF, Del Bianchi VL (2022) Optimization of agro-industrial coproducts (molasses and cassava wastewater) for the sim ultaneous production of lipids and carotenoids by Rhodotorula mucilaginosa. Biocatal Agric Biotechnol 42:102342. https://doi.org/10.1016/j.bcab.2022.102342

Macheix JJ, Fleuriet A, Billot J (1990) Fruit phenolics. CRC Press, Boca Raton. In: Sarni-Manchado P, Cheynier V (eds) Les Polyphénols en Agroalimentaire. Tec et Doc Lavoisier, Paris (2006)

Madej A, Popłoński J, Huszcza E (2012) Transformations of naringenin by yeast *Rhodotorula marina*. Przem Chem 91:856–859

Madej A, Popłoński J, Huszcza E (2014) Improved oxidation of naringenin to carthamidin and isocarthamidin by *Rhodotorula marina*. Appl Biochem Biotechnol 173:67–73. https://doi.org/10.1007/s12010-014-0787-4

Mahajan S, Nikhanj P, Singh A (2024) Microbial biopigment production by *Rhodotorula* sp. using horticultural waste; its functionality, stability and applications. Indian J Microbiol. https://doi.org/10.1007/s12088-024-01370-y

Mandelli F, Miranda VS, Rodrigues E, Mercadante AZ (2012) Identification of carotenoids with high antioxidant capacity produced by extremophile microorganisms. World J Microbiol Biotechnol 28(4):1781–1790. https://doi.org/10.1007/s11274-011-0993-y

Mantzouridou F, Roukas T, Kotzekidou P (2002) Optimization of β-carotene production from synthetic medium by *Blakeslea trispora* in a stirred tank reactor and relationship between morphological changes and pigment formation. Food Biotechnol 16:167–187. https://doi.org/10.1081/fbt-120016666

Markou G, Chatzipavlidis I, Georgakakis D (2012) Cultivation of *Arthrospira* (*Spirulina*) *platensis* in olive-oil mill wastewater treated with sodium hypochlorite. Bioresour Technol 112:234–241. https://doi.org/10.1016/j.biortech.2012.02.098

Martin D, Amado AM, Gonzálvez AG, Marques MPM, Batista de Carvalho LAE, González Ureña A (2019) FTIR spectroscopy and DFT calculations to probe the kinetics of β-carotene thermal degradation. J Phys Chem A 123:5266–5273. https://doi.org/10.1021/acs.jpca.9b02327

Martínez-Cruz A, Fernandes A, Ciríaco L, Pacheco MJ, Carvalho F, Afonso A, Madeira L, Luz S, Lopes A (2020) Electrochemical oxidation of effluents from food processing industries: a short review and a case-study. Water 12:3546. https://doi.org/10.3390/w12123546

Martinez-Garcia G, Johnson AC, Bachmann RT, Williams CJ, Burgoyne A, Edyvean RGJ (2007) Two-stage biological treatment of olive mill wastewater with whey as co-substrate. Int Biodeterior Biodegrad 59(4):273–282. https://doi.org/10.1016/j.ibiod.2007.03.008

Mast B, Zöhrens N, Schmidl F, Hernandez R, French WT, Merkt N, Claupein W, Graeff-Hönninger S (2014) Lipid production for microbial biodiesel by the oleaginous yeast *Rhodotorula glutinis* using hydrolysates of wheat straw and *Miscanthus* as carbon sources. Waste Biomass Valorization 5:955–962. https://doi.org/10.1007/s12649-014-9312-9

Maza MA, Martínez JM, Delso C, Camargo A, Raso J, Álvarez I (2020) PEF-dependency on polyphenol extraction during maceration/fermentation of Grenache grapes. Innov Food Sci Emerg Technol 60:102303. https://doi.org/10.1016/j.ifset.2020.102303

Meléndez-Martínez AJ, Esquivel P, Rodriguez-Amaya DB (2023) Comprehensive review on carotenoid composition: transformations during processing and storage of foods. Food Res Int 169:112773. https://doi.org/10.1016/j.foodres.2023.112773

Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428. https://doi.org/10.1021/ac60147a030

Mohiuddin O, Harvey AP, Orta Ledesma MT, Velasquez-Orta S (2024) Bioremediation of waste by yeast strains. Electron J Biotech 69:30–42. https://doi.org/10.1016/j.ejbt.2024.01.005

Mordi RC, Ademosun OT, Ajanaku CO, Olanrewaju IO, Walton JC (2020) Free radical mediated oxidative degradation of carotenes and xanthophylls. Molecules 25(5):1038. https://doi.org/10.3390/molecules25051038

Morillo JA, Antizar-Ladislao B, Monteoliva-Sánchez M, Ramos-Cormenzana A, Russell NJ (2009) Bioremediation and biovalorisation of olive-mill wastes. Appl Microbiol Biotechnol 82:25–39. https://doi.org/10.1007/s00253-008-1801-y

Mtui GYS (2012) Lignocellulolytic enzymes from tropical fungi. Sci Res Essays 7(15):1544–1555. https://doi.org/10.5897/sre11.1812

Mussagy CU, Santos-Ebinuma VC, Kurnia KA, Dias ACRV, Carvalho P, Coutinho JAP, Pereira JFB (2020) Integrative platform for the selective recovery of intracellular carotenoids and lipids from *Rhodotorula glutinis* CCT-2186 yeast using mixtures of bio-based solvents. Green Chem 22:8478–8494. https://doi.org/10.1039/D0GC02992K

Nazos T, Stratigakis NC, Spantidaki M, Spantidaki AL, Ghanotakis DF (2023) Characterization of cheese whey effluents and investigation of their potential to be used as a nutrient substrate for *Chlorella* biomass production. Waste Biomass Valor 14:3643–3655. https://doi.org/10.1007/s12649-023-02081-z

Nunes DD, Pillay VL, Van Rensburg E, Pott RWM (2024) Oleaginous microorganisms as a sustainable oil source with a focus on downstream processing and cost-lowering production strategies: a review. Bioresour Technol Rep 26:101871. https://doi.org/10.1016/j.biteb.2024.101871

OECD/FAO (2022) OECD-FAO agricultural outlook 2022–2031. OECD Publishing, Paris. https://doi.org/10.1787/f1b0b29c-en

Ogg CL (1960) Determination of nitrogen by the micro-Kjeldahl method. J AOAC Int 43(3):689–693. https://doi.org/10.1093/jaoac/43.3.689

Olmos J, Acosta M, Mendoza G, et al. (2020) *Bacillus subtilis*, an ideal probiotic bacterium for shrimp and fish aquaculture that increases feed digestibility, prevents microbial diseases, and reduces water

pollution. Arch Microbiol 202:427–435. https://doi.org/10.1007/s00203-019-01757-2

Otero P, Garcia-Oliveira P, Carpena M, Barral-Martinez M, Chamorro F, Echave J, Garcia-Perez P, Cao H, Xiao J, Simal-Gandara J, Prieto MA (2021) Applications of by-products from the olive oil processing: revalorization strategies based on target molecules and green extraction technologies. Trends Food Sci Technol 116:1084–1104. https://doi.org/10.1016/j.tifs.2021.09.007

Ozcelik D, Suwal S, Ray C, Tiwari BK, Jensen PE, Poojary MM (2024) Valorization of dairy side-streams for the cultivation of microalgae for value added food products. Trends Food Sci Technol 146:104386. https://doi.org/10.1016/j.tifs.2024.104386

Palupi E, Nasir SQ, Jayanegara A, Susanto I, Ismail A, Iwansyah AC, Setiawan B, Sulaeman A, Damanik MRM, Filianty F (2025) Meta-analysis on the fatty acid composition of edible insects as a sustainable food and feed. Future Foods 11:100529. https://doi.org/10.1016/j.fufo.2024.100529

Paz A, Zerva A, Topakas E (2023) Evaluation of olive mill wastewater as culture medium to produce lipolytic enzymes by *Bacillus aryabhattai* BA03. Biocatal Agric Biotechnol 48:102643. https://doi.org/10.1016/j.bcab.2023.102643

Petrotos KB, Kokkora MI, Papaioannou C, Gkoutsidis PE (2016) Olive mill wastewater concentration by two-stage reverse osmosis in tubular configuration, in a scheme combining open and tight membranes. Desalin Water Treat 57(44):20621–20630. https://doi.org/10.1080/19443994.2015.1123198

Phan A, Mijar S, Harvey C, Biswas D (2025) *Staphylococcus aureus* in foodborne diseases and alternative intervention strategies to overcome antibiotic resistance by using natural antimicrobials. Microorganisms 13(8):732. https://doi.org/10.3390/microorganisms13081732

Piccinin E, Cariello M, De Santis S, Ducheix S, Sabbà C, Ntambi JM, Moschetta A (2019) Role of oleic acid in the gut-liver axis: from diet to the regulation of its synthesis via stearoyl-CoA desaturase 1 (SCD1). Nutrients 11:2283. https://doi.org/10.3390/nu11102283

Prazeres AR, Carvalho F, Rivas J (2012) Cheese whey management: a review. J Environ Manage 110:48–68. https://doi.org/10.1016/j.jenvman.2012.05.018

Quijano-Ortega N, Fuenmayor CA, Zuluaga-Dominguez C, Diaz-Moreno C, Ortiz-Grisales S, García-Mahecha M, Grassi S (2020) FTIR-ATR spectroscopy combined with multivariate regression modeling as a preliminary approach for carotenoids determination in *Cucurbita* spp. Appl Sci 10:3722. https://doi.org/10.3390/app10113722

Raita S, Kusnere Z, Spalvins K, Blumberga D (2022) Optimization of yeast cultivation factors for improved SCP production. Environ Clim Technol 26:848–861. https://doi.org/10.2478/rtuect-2022-0064

Rasouli H, Hosseini-Ghazvini SMB, Adibi H, Khodarahmi R (2017) Differential α-amylase/α-glucosidase inhibitory activities of plant-derived phenolic compounds: a virtual screening perspective for the treatment of obesity and diabetes. Food Funct 8:1942–1954. https://doi.org/10.1039/C7FO00220C

Ratledge C (1994) Yeasts, moulds, algae and bacteria as sources of lipids. In: Kamel S, Kakuda Y (eds) Technological Advances in Improved and Alternative Sources of Lipids. Blackie Academic and Professional, London, pp 239. https://doi.org/10.1007/978-1-4615-2109-9_9

Renda G, Sari S, Barut B, Šoral M, Liptaj T, Korkmaz B, Özel A, Erik I, Şöhretoğlu D (2018) A-Glucosidase inhibitory effects of polyphenols from *Geranium asphodeloides*: inhibition kinetics and mechanistic insights through *in vitro* and *in silico* studies. Bioorg Chem 81:545–552. https://doi.org/10.1016/j.bioorg.2018.09.009

Řezanka T, Víden I, Go JV, Dor I, Dembitsky VM (2003) Polar lipids and fatty acids of three wild cyanobacterial strains of the genus *Chroococcidiopsis*. Folia Microbiol 48:781–786. https://doi.org/10.1007/BF02931514

Ribeiro JES, Sant' Ana AMS, Martini M, Sorce C, Andreucci A, Nóbrega de Meloe DJ, da Silva FLH (2019) *Rhodotorulaglutinis* cultivation on cassava wastewater for carotenoids and fatty acids generation. Biocatal Agric Biotechnol 22:101419

Ribeiro RMMGP, Picão BW, Gonçalves DO, Scontri M, Mazziero VT, Mussagy CU, Raghavan V, Astudillo-Castro C, Córdova A, Cerri MO, Tambourgi EB (2023) Synergistic effects of stirring and aeration rate on carotenoid production in yeast *Rhodotorula toruloides* CCT 7815 envisioning their application as soap additives. Fermentation 9(9):828. https://doi.org/10.3390/fermentation9090828

Rodriguez-Amaya DB, Carle R (2021) Alterations of natural pigments. In: Rodriguez-Amaya DB, Amaya-Farfan J (eds) Chemical Changes During Processing and Storage of Foods. Elsevier Academic Press, London, pp 265–327. https://doi.org/10.1016/B978-0-12-817380-0.00007-5

Rubio-Diaz DE, De Nardo T, Santos A, de Jesus S, Francis D, Rodriguze-Saona LE (2010) Profiling of nutritionally important carotenoids from genetically diverse tomatoes by infrared spectroscopy. Food Chem 120:282–289. https://doi.org/10.1016/j.foodchem.2009.09.060

Saenge C, Cheirsilp B, Suksaroge TT, Bourtoom T (2011) Potential use of oleaginous red yeast *Rhodotorula glutinis* for the bioconversion of crude glycerol from biodiesel plant to lipids and carotenoids. Process Biochem 46:210–218. https://doi.org/10.1016/j.procbio.2010.08.009

Sakamoto T, Murata N (2002) Regulation of the desaturation of fatty acids and its role in tolerance to cold and salt stress. Curr Opin Microbiol 5(2):206–210. https://doi.org/10.1016/s1369-5274(02)00306-5

Salar RK, Certik M, Brezova V, Brlejova M, Hasunova V, Breierová E (2012) Stress influenced increase in phenolic content and radical scavenging capacity of Rhodotorula glutinis CCY 20–2–26. 3 Biotech 3(1):53–60. https://doi.org/10.1007/s13205-012-0069-1

Sandhiya L, Zipse H (2022) Conformation-dependent antioxidant properties of β-carotene. Org Biomol Chem 20(1):152–162. https://doi.org/10.1039/d1ob01723c

Schlücker S, Szeghalmi A, Schmitt M, Popp J, Kiefer W (2003) Density functional and vibrational spectroscopic analysis of β-carotene. J Raman Spectrosc 34:413–419. https://doi.org/10.1002/jrs.1013

Schneider T, Graeff-Hönninger S, French WT, Hernandez R, Merkt N, Claupein W, Hetrick M, Pham P (2013) Lipid and carotenoid production by oleaginous red yeast *Rhodotorula glutinis* cultivated on brewery effluents. Energy 61:34–43. https://doi.org/10.1016/j.energy.2012.12.026

Sebastian S, Tripathi AD, Paul V, Darani KK, Agarwal A (2023) Production kinetics and characterization of natural food color (torularhodin) with antimicrobial potential. Bioresour Technol Rep 24:101652. https://doi.org/10.1016/j.biteb.2023.101652

Šeregelj V, Estivi L, Brandolini A, Ćetković G, Šaponjac VT, Hidalgo A (2022) Kinetics of carotenoids degradation during the storage of encapsulated carrot waste extracts. Molecules 27:8759. https://doi.org/10.3390/molecules27248759

Sereti F, Alexandri M, Papadaki A, Papapostolou H, Kopsahelis N (2024) Carotenoids production by *Rhodosporidium paludigenum* yeasts: characterization of chemical composition, antioxidant and antimicrobial properties. J Biotechnol 386:52–63. https://doi.org/10.1016/j.jbiotec.2024.03.011

Sereti F, Papadaki A, Alexandri M, Kachrimanidou V, Kopsahelis N (2023) Exploring the potential of novel *R. kratochvilovae* red yeasts towards the sustainable synthesis of natural carotenoids. Sustain Chem Pharm 31:100927. https://doi.org/10.1016/j.scp.2022.100927

Shahawy AE, Ahmed IA, Nasr M, Ragab AH, Al-Mhyawi SR, Elamin KMA (2021) Organic pollutants removal from olive mill wastewater using electrocoagulation process via central composite design (CCD). Water 13:3522. https://doi.org/10.3390/w13243522

Sharma R, Ghoshal G (2020) Optimization of carotenoids production by *Rhodotorula mucilaginosa* (MT CC 1403) using agro-industrial waste in bioreactor: a statistical approach. Biotechnol Rep 25:e00407. https://doi.org/10.1016/j.btre.2019.e00407

Sineli PE, Maza DD, Aybar MJ, Figueroa LIC, Viñarta SC (2022) Bioconversion of sugarcane molasses and waste glycerol on single cell oils for biodiesel by the red yeast *Rhodotorula glutinis* R4 from Antarctica. Energy Convers Manag X 16:100331. https://doi.org/10.1016/j.ecmx.2022.100331

Somashekar D, Joseph R (2000) Inverse relationship between carotenoid and lipid formation in *Rhodotorula gracilis* according to the C/N ratio of the growth medium. World J Microbiol Biotechnol 16:491–493. https://doi.org/10.1023/A:1008917612616

Sordon S, Madej A, Popłoński J, Bartamańska A, Tronina T, Brzezowska E, Juszczyk P, Huszcza E (2016) Regioselective ortho-hydroxylations of flavonoids by yeast. J Agric Food Chem 64(27):5630–5637. https://doi.org/10.1021/acs.jafc.6b02210

Souilem S, El-Abbassi A, Kiai H, Hafidi A, Sayadi S, Galanakis CM (2017) Olive oil production sector: environmental effects and sustainability challenges. In: Olive Oil: Chemistry and Technology, Elsevier, pp 1–28. https://doi.org/10.1016/B978-0-12-805314-0.00001-7

Stahl W, Sies H (2003) Antioxidant activity of carotenoids. Mol Aspects Med 24(6):345–351. https://doi.org/10.1016/S0098-2997(03)00030-X

Susamci E, Romero C, Tuncay O, Brenes M (2017) An explanation for the natural debittering of Hurma olives during ripening on the tree. Grasas Aceites 68:182. https://doi.org/10.3989/gya.1161162

Taskin M, Ucar MH, Unver Y, Kara AA, Ozdemir M, Ortucu S (2016) Lipase production with free and immobilized cells of cold-adapted yeast *Rhodotorula glutinis* HL25. Biocatal Agric Biotechnol 8:97–103. https://doi.org/10.1016/j.bcab.2016.08.009

Tkáčová J, Klempová T, Čertík M (2018) Kinetic study of growth, lipid and carotenoid formation in β-carotene producing *Rhodotorula glutinis*. Chem Pap 72:1193–1203. https://doi.org/10.1007/s11696-017-0368-4

Uemura H (2012) Synthesis and production of unsaturated and polyunsaturated fatty acids in yeast: current state and perspectives. Appl Microbiol Biotechnol 95:1–12. https://doi.org/10.1007/s00253-012-4105-1

Valdes O, Ramirez C, Perez F, Garcia-Vicencio S, Nosaka K, Penailillo L (2021) Contralateral effects of eccentric resistance training on immobilized arm. Scand J Med Sci Sports 31:76–90. https://doi.org/10.1111/sms.13821

Vlietinck AJ, Vanden Berghe DA (1991) Can ethnopharmacology contribute to the development of antiviral drugs? J Ethnopharmacol 32(1–3):141–153. https://doi.org/10.1016/0378-8741(91)90112-q

Wang M, Mao W, Wang X, et al. (2019) Efficient simultaneous production of extracellular polyol esters of fatty acids and intracellular lipids from inulin by a deep-sea yeast *Rhodotorula paludigena* P4R5. Microb Cell Fact 18:149. https://doi.org/10.1186/s12934-019-1200-3

Wang Z-P, Fu W-J, Xu H.-M, Chi Z-M (2014) Direct conversion of inulin into cell lipid by an inulinase-producing yeast *Rhodosporidium toruloides* 2F5. Bioresour Technol 161:131–136. https://doi.org/10.1016/j.biortech.2014.03.038

Weng LC, Pasaribu B, Lin IP, Tsai CH, Chen CS, Jiang PL (2014) Nitrogen deprivation induces lipid droplet accumulation and alters fatty acid metabolism in symbiotic dinoflagellates isolated from *Aiptasia pulchella*. Sci Rep 4:5777. https://doi.org/10.1038/srep05777

Xiao Y, Zan F, Zhang W, Hao T (2022) Alleviating nutrient imbalance of low carbon-to-nitrogen ratio food waste in anaerobic digestion by controlling the inoculum-to-substrate ratio. Bioresour Technol 346:126342. https://doi.org/10.1016/j.biortech.2021.126342

Yahia EM, Ornelas-Paz JJ, Emanuelli T, Jacob-Lopes E, Queiroz Zepka L, Cervantes-Paz B (2017) Chemistry, stability, and biological actions of carotenoids. In: Yahia EM (ed) Fruit and Vegetable Phytochemicals, 2nd edn. Wiley, pp. 285–346. https://doi.org/10.1002/9781119158042.ch15

Yen HW, Hu CY, Liang WS, (2019) A cost-efficient way to obtain lipid accumulation in the oleaginous yeast *Rhodotorula glutinis* using supplemental waste cooking oils (WCO). J Taiwan Inst Chem Eng 97:80–87. https://doi.org/10.1016/j.jtice.2019.02.012

Yildirim A, Mavi A, Kara AA (2001) Determination of antioxidant and antimicrobial activities of Rumex crispus L. extracts. J Agric Food Chem 49(8):4083–4089. https://doi.org/10.1021/jf0103572

Yimyoo T, Yongmanitchai W, Limtong S (2011) Carotenoid production by *Rhodosporidium paludigenum* DMKU3-LPK4 using glycerol as the carbon source. Agric Nat Resour 45:90–100. https://li01.tci-thaijo.org/index.php/anres/article/view/245137

Zahi MR, Zam W, El Hattab M (2022) State of knowledge on chemical, biological, and nutritional properties of olive mill wastewater. Food Chem 381:132238. https://doi.org/10.1016/j.foodchem.2022.132238

Zavra A, Hatzigiannakis E, Oikonomou EK, Guitonas A (2024) Biodegradation of olive mill wastewater by two bacterial strains. Desalin Water Treat 317:100180. https://doi.org/10.1016/j.dwt.2024.100180

Zhang G, French WT, Hernandez R, Alley E (2011) Effects of furfural and acetic acid on growth and lipid production from glucose and xylose by *Rhodotorula glutinis*. Biomass Bioenergy 35:734–740. https://doi.org/10.1016/j.biombioe.2010.10.009

Zhao CH, Chi Z, Zhang F, Guo FJ, Li M, Song WB, Chi ZM (2011) Direct conversion of inulin and extract of tubers of Jerusalem artichoke into single cell oil by co-cultures of *Rhodotorula mucilaginosa* TJY15a and immobilized inulinase-producing yeast cells. Bioresour Technol 102:6128–6133. https://doi.org/10.1016/j.biortech.2011.02.077

Zhao CH, Zhang T, Li M, Chi ZM (2010) Single cell oil production from hydrolysates of inulin and extract of tubers of Jerusalem artichoke by *Rhodotorula mucilaginosa* TJY15a. Process Biochem 45:1121–1126. https://doi.org/10.1016/j.procbio.2010.04.002

Zhao D, Li C (2022) Multi-omics profiling reveals potential mechanisms of culture temperature modulating biosynthesis of carotenoids, lipids, and exopolysaccharides in oleaginous red yeast *Rhodotorula glutinis* ZHK. LWT 171:114103. https://doi.org/10.1016/j.lwt.2022.114103

Zhou L, Tong H, Tang H, Pang S (2021) Fatty acid desaturation is essential for *C. elegans* longevity at high temperature. Mech Ageing Dev 200:111586. https://doi.org/10.1016/j.mad.2021.111586

Zoz L, Carvalho JC, Soccol VT, Casagrande TC, Cardoso L (2015) Torularhodin and torulene: bioproduction, properties and prospective applications in food and cosmetics – a review. Braz Arch Biol Technol 58(2):278. https://doi.org/10.1590/S1516-89132014000152

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