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Red torulene from yeast: Isolation, characterization, bioprocess development, and its underexplored pioneering application as a natural food colorant

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ABSTRACT

Torulene, a red carotenoid produced by *Rhodotorula* species, has gained significant attention for its vibrant color and potential health benefits. However, while several reviews have explored its properties, there remains a lack of research on its practical applications across diverse industries. This study aims to fill this gap by isolating and characterizing a novel *R. mucilaginosa* strain (PUCV 645) from Chile, with a focus on evaluating the use of edible oils as extraction agents for the recovery of torulene-rich extracts (TRE). The COSMO-SAC predictive model was employed to explain the interactions involved in the green extraction process. Additionally, TRE has two innovative applications: its use as a natural food colorant in pasta and as a component in colored vegan mayonnaise, where it is combined with residual aquafaba as a potential egg replacer. Through molecular identification, phylogenetic analysis, and carotenoid characterization, this work provides the first comprehensive exploration of *R. mucilaginosa* PUCV 645's potential for food industrial applications. The findings highlight the promise of TRE as a sustainable, natural alternative in food manufacturing, paving the way for its integration into the food industry. This study also contributes to the understanding torulene's versatility and potential market applications.

1. Introduction

In recent decades, biotechnological advancements have underscored the significant potential of microorganisms as underexplored sources of novel bioactive natural pigments (Mussagy et al., 2023; N. Sharma et al., 2024). The search for novel microbial sources addresses the need for innovative solutions to challenges like food colorants, preservation, health enhancement, and sustainable practices. Exploring new strains is key to identifying unique pigments such as carotenoids with high-value applications (i.e., colorants), particularly in food manufacturing (Dufossé, 2024). Yeasts from genera *Rhodotorula* are of particular interest due to their ability to biosynthesize carotenoids including torulene, β-carotene and γ-carotene (Fig. 1). Beyond the general importance of carotenoids in the acceptability of foods due to their

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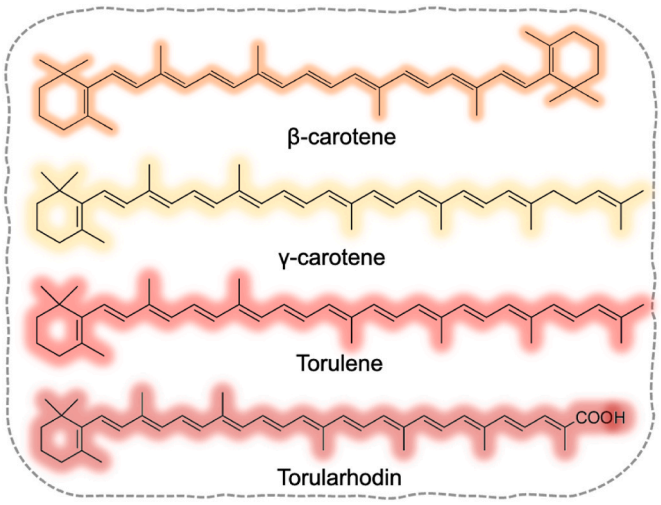

Fig. 1. Chemical structures of major *Rhodotorula mucilaginosa* carotenoids.

colorant properties, β-carotene is a provitamin A carotenoid that has been associated to diverse health benefits. γ-carotene is a little studied provitamin A carotenoid that contains only an unsubstituted β-ring, unlike β-carotene that exhibits two such rings (Fig. 1) (Meléndez-Martínez et al., 2007, 2022). Torulene is a little studied carotenoid of fungal origin. Evidence is accumulating that it can exhibit interesting activities, not to mention that, having also an unsubstituted β-ring, it could also exhibit vitamin A activity (Kot et al., 2019, 2020; Mussagy et al., 2019, 2020; Statzell-Tallman & Fell, 1998). The number of conjugated double bonds (13) of torulene is superior compared to that of typical dietary carotenoids, such as β-carotene or lycopene (both with 11 conjugated double bonds). Since the unsaturation level of carotenoids is much related to properties including light absorption, color, shape or antioxidant capacity, there is no doubt that this carotenoid is amenable for the development of carotenoid-based innovative products, one of the opportunities envisaged by the COST Action EUROCAROTEN (https://www.cost.eu/actions/CA15136/#tabs|Name:overview) (Meléndez-Martínez et al., 2007, 2022). Torulene has been reported to be produced by several aquatic yeasts, the exploitation of which is especially interesting in the current scenario of promotion of the Blue Economy (Mapelli-Brahm et al., 2023).

Natural carotenoids derived from *Rhodotorula* species, particularly *R. glutinis* and *R. mucilaginosa*, are increasingly recognized as promising alternatives to synthetic dyes in food applications (Martínez et al., 2020; R. Sharma & Ghoshal, 2021). Despite being known for over a century, the commercialization of *Rhodotorula* sp. as microbial cell factories for natural carotenoids has yet to be realized (Mussagy, Ribeiro, et al., 2022c). This delay can be attributed to several factors: *(i)* low productivity lab scale and the limited availability of large-scale production knowledge; *(ii)* insufficient technical-economic assessments; and *(iii)* lack of well-established production processes (Mussagy, Ribeiro, et al., 2022c). Additionally, the strong market presence of *Blakeslea trispora* for β-carotene production and the established industrial processes for astaxanthin production by microalgae *Haematococcus* and bacteria *Paracoccus* further hinder the adoption of *Rhodotorula* sp. in the market (Ariyadasa et al., 2024; Bampidis et al., 2022; Du et al., 2015, 2017; EFSA, 2007; Moliné et al., 2010; Sakaki et al., 2001; Turck et al., 2020). Consequently, these challenges contribute to underutilizing *Rhodotorula*-based carotenes and xanthophylls in commercial applications. However, *R. mucilaginosa* are capable of producing relatively unexplored carotenoids, such as torulene and torularhodin, which have various biological applications, including antioxidant activity (Du et al., 2017; Jin et al., 2024; Kot et al., 2018; Mussagy, Gonzalez-Miquel, et al., 2022b). Although torulene has demonstrated promising antioxidant and anti-inflammatory properties, cytotoxicity remains a critical concern for food-grade applications, comprehensive toxicological including cytotoxicity and genotoxicity assays are required. It is important to note that the production of these carotenoids (e.g., torulene) is not intended to replace existing market options products in the market; instead, they aim to provide consumers with more options (Mussagy et al., 2024).

The literature highlights a 'significant gap' in application-focused research on torulene, particularly regarding its potential as a natural and vibrant pigment in food products, its potent antioxidant properties in cosmetics, and its health-promoting benefits in nutraceuticals (Jin et al., 2024). Bridging this gap would expand our understanding of microbial-based torulene and drive its production, commercialization, and adoption across diverse industries. Advancing the field of natural torulene production requires identifying new microbial sources with metabolic pathways optimized for its biosynthesis, alongside refining *upstream* and *downstream* processes. *Upstream* improvements include selecting high-yielding organisms, optimizing production processes, and utilizing low-cost industrial by-products as nutrient sources. At the same time, *downstream* advancements focus on sustainable and integrative methods to develop innovative torulene-rich additives that address the increasing consumer demand for natural and sustainable alternatives.

Given the promising potential of *Rhodotorula* sp. in the food industry, this study aimed to: *(1)* isolate highly carotenogenic *Rhodotorula* sp. strains with potential commercial relevance from the Valparaíso-Chile environment, *(2)* identify the isolates at the species level using molecular techniques, *(3)* characterize torulene-rich extracts (TRE), and *(4)* evaluate the use of edible oils as extraction agents envisioning the integration in food applications; at this stage the COSMO-SAC predictive model was employed to explain the solute-solvent interactions in the extraction process. Edible oils were selected as extractants due to their hydrophobic nature, high compatibility with food matrices. Their ability to solubilize carotenoids while functioning as both solvents and carriers allows for direct incorporation into foods without additional purification steps, aligning with green extraction principles and clean-label product development. Furthermore, the study investigated the stability of TRE-enriched oils for direct application in food products, exploring two specific applications: *(i)* enhancing the color of pasta using TRE and *(ii)* combining TRE with residual aquafaba as a potential egg replacer for colored vegan mayonnaise. Note that, aquafaba, the residual derived from legumes such as chickpeas, has emerged as a promising substitute for egg whites due to its emulsifying properties, which are mainly attributed to proteins (such as legumin and vicilin-like proteins) and polysaccharides (Huang et al., 2024). For clarity, TRE (torulene-rich extract), is referred to the pigment-rich concentrate obtained directly from biomass, and TRE-enriched oils, are formulations where TRE has been incorporated into a oil matrix.

2. Experimental procedure

2.1. Materials

A high-purity β-carotene standard (≥97 %) was procured from Sigma-Aldrich (St. Louis, MO, USA). Edible oils, including canola, sunflower, and corn oils, were sourced from the Cencosud Group (Santiago, Chile). High-performance liquid chromatography (HPLC)-grade solvents, also obtained from Sigma-Aldrich, were used for all HPLC analyses. Other solvents and chemicals used in the study were of analytical grade.

2.2. Microorganism and culture media

The analysis was conducted at the Laboratorio de Fitopatología in the Escuela de Agronomía, Pontificia Universidad Católica de Valparaíso (PUCV). Tomato plants containing *Rhodotorula mucilaginosa* were collected from a polyethylene greenhouse owned by the Escuela de Agronomía. To isolate and cultivate the microorganism, three types of culture media were utilized at different stages: *(i)* YM broth (liquid)

medium was used for the initial isolation of the strain and was prepared by combining: 3 g/L of malt extract, 3 g/L of yeast extract, 5 g/L of peptone, and 20 g/L of glucose. *(ii)* 523 culture medium was used for the isolated strain, prepared using 10 g/L of sucrose, 8 g/L of hydrolyzed casein, 4 g/L of yeast extract, 2 g/L of dipotassium phosphate, 0.3 g/L of magnesium sulfate heptahydrate, and 15 g/L of granulated agar in distilled water. *(iii)* YPD culture medium was formulated for subsequent cultivation and maintenance of the strain and was prepared by combining 20 g/L of peptone, 20 g/L of glucose, 10 g/L of yeast extract, and 15 g/L of granulated agar in distilled water. All culture media were sterilized at 121 °C for 21 min. Once cooled to approximately 45 °C, 20 mL of each medium was poured into 90 mm diameter Petri dishes, and separate plates were prepared for each type of culture medium.

2.3. Isolation, morphological and biochemical characterization of PUCV 645 strain

A phytopathological analysis was conducted on the tomato plants. Leaf and stem samples (1 cm^2) were collected and triple-washed with sterile distilled water to remove surface contaminants. On a sterile surface, the samples were crushed using a glass rod and macerated for 3 min with 1 mL of sterile distilled water. The resulting macerate was streaked onto Petri dishes containing a "523″ culture medium using the streak plate technique to isolate individual colonies. After 48 h of incubation at 26 °C, orange to pink globose colonies with defined edges were observed. These colonies were subsequently transferred to the YPD culture medium for purification using the same streaking method. After 48 h of incubation at 26 °C, purified colonies were subjected to biochemical characterization, including Gram staining, cytochrome oxidase, and catalase tests (Alhazmi & Alshehri, 2025).

2.4. Identification of R. mucilaginosa

A purified colony was grown overnight at 26 °C in a YPD liquid medium. DNeasy Blood and Tissue Kit (Qiagen, Germany) was used for the DNA extraction following the manufacturer's instructions. ITS-5.8S-ITS1 region was amplified using the pair primers ITS1-ITS4 (White et al., 1990). The PCR reaction consisted of the following: 12.5 μL SapphireAmp® Fast PCR Master Mix (2x); 0.4 μM of each primer; 1 μL template DNA and H_2O to a final volume of 22 μL. DNA was amplified using 30 cycles of denaturation to 98 °C × 5 s, annealing 55 °C × 5 s, and final elongation 72 °C × 10 s. The PCR product was visualized in an electrophoresis gel (1 %) staining with GelRed (Biotium) and viewing with UV transilluminator (MaestroGen). PCR product was sequenced by Macrogen (Santiago, Chile), assembled, and edited using Geneious 10.0.6 software. Using the BLAST tool, the obtained sequence was compared against reference sequences from the National Center of Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov).

2.5. Phylogenetic analysis isolate PUCV 645

A multi-locus phylogenetic analysis was performed using Maximum Parsimony (MP) in MEGAX (Kumar et al., 2018) using Bisection and Reconnection algorithms, bootstrap values were calculated using 1000 replicates yielding the MP tree. The tree was rooted with *Nakaseomyces glabratus* NRRL—Y65 and other reference isolates of *Rhodotorula mucilaginosa*. The tree length, retention index, rescaled consistency index, and homoplasy index were calculated automatically in MEGA X.

2.6. Carotenoids production and total extraction

The *R. mucilaginosa* isolate PUCV 645 was cultivated in 100 mL Erlenmeyer flasks containing 20 mL YPD medium, and incubated at 26 °C with continuous shaking at 170 rpm for 48 h. After incubation, the biomass was collected, washed with phosphate buffer (pH 7.0), and dried at 50 °C in the absence of direct light to reduce degradation for 24 h to prepare for carotenoid extraction and quantification. For carotenoid extraction, the dried biomass was subjected to a sequential extraction of maceration process using 5 mL of acetone per round until the biomass became bleached. After extraction, the colored mixtures were centrifuged at 2500×*g* for 10 min at 25 °C using a Minicen Orto Alresa centrifuge (Madrid, Spain). The resulting-colored supernatant, which contained torulene-enriched extracts, was carefully collected and pooled. The pooled volume was then concentrated to 2 mL under vacuum. Finally, the concentrated extract was filtered through 0.45 μm filters before being subjected to High-Performance Liquid Chromatography (HPLC) analysis for carotenoid characterization and total carotenoid quantification (Vanessa Caicedo-Paz et al., 2024).

2.7. Carotenoid extraction with edible oils

R. mucilaginosa isolate PUCV 645 biomass was weighed (0.2 g) in a 10 mL dark glass tube and added to edible oils (2 mL). After stirring (300 rpm) at 80 °C for 30 min, the sample solution was centrifuged (2500×*g* for 10 min at 25 °C) to separate the oil from the residual biomass and then subjected to analysis. The total carotenoid contents in the extracts with each edible oil were analyzed using the methodology previously described } (Sachindra & Mahendrakar, 2005).

2.8. HPLC-PDA-MS analysis of individual carotenoids

The carotenoids characterization was carried out by high performance liquid chromatography, using a Shimadzu (Kyoto, Japan) Nexera X2 instrument, equipped with two binary solvent pumps LC-30 AD, a DGU-20A5R degassing unit, a CTO-20AC column oven, a SIL-30AC autosampler and a photodiode array detector (PDA) SPD-M30A serially coupled to an LCMS-2020 spectrometer via an atmospheric pressure chemical ionization (APCI) source operating in both positive and negative ionization mode. Separations were performed on a C18 column (Supelco, 10 cm × 2.1 mm, 2.7 μm particles size). The mobile phases were Acetonitrile (solvent A) and Isopropanol (solvent B), applying the following gradient: 5–70 % B in 40 min. UV–visible spectra were recorded between 200 and 700 nm, and chromatograms were monitored at 450 nm (sampling rate: 4.1667 Hz; time constant: 0.480 s). Mass spectrometry (MS) parameters for both positive and negative APCI modes were as follows: *m/z* range 10–700 amu, scan speed 625 u/s, nebulizing gas (N_2) flow rate 3 L/min, event time 1 s, interface temperature 350 °C, DL (desolvation line) temperature 300 °C, heat block temperature 300 °C, and drying gas flow 15 mL/min. Data were processed using Labsolution version 5.10.153 (Shimadzu, Milan, Italy), and compounds were identified based on their chromatographic and spectroscopic properties.

2.9. COSMO-SAC prediction of carotenoid solvation by fatty acids

For the qualitative assessment of the fatty acids effect in the solvation of torulene found in *R. mucilaginosa* isolate PUCV 645 biomass the COSMO-SAC (Conductor-like Screening Model - Segment Activity Coefficient) model was applied following the procedure previously described (Goltz et al., 2021; Mussagy, Farias, et al., 2022a). The COSMO-SAC model is a thermodynamic approach used to predict how molecules interact in mixtures, particularly in terms of solubility and solvation behavior. It is based on quantum chemical calculations and considers the surface charge distribution of molecules to estimate activity coefficients in various solvents (Goltz et al., 2021; Mussagy, Farias, et al., 2022a).

2.10. Colorimetric method and torulene-rich extracts stability

Color measurements of the samples were quantified using the CIELab color space, which is defined by three components: lightness (L*), the red-green value (a*), and the blue-yellow value (b*). The total color

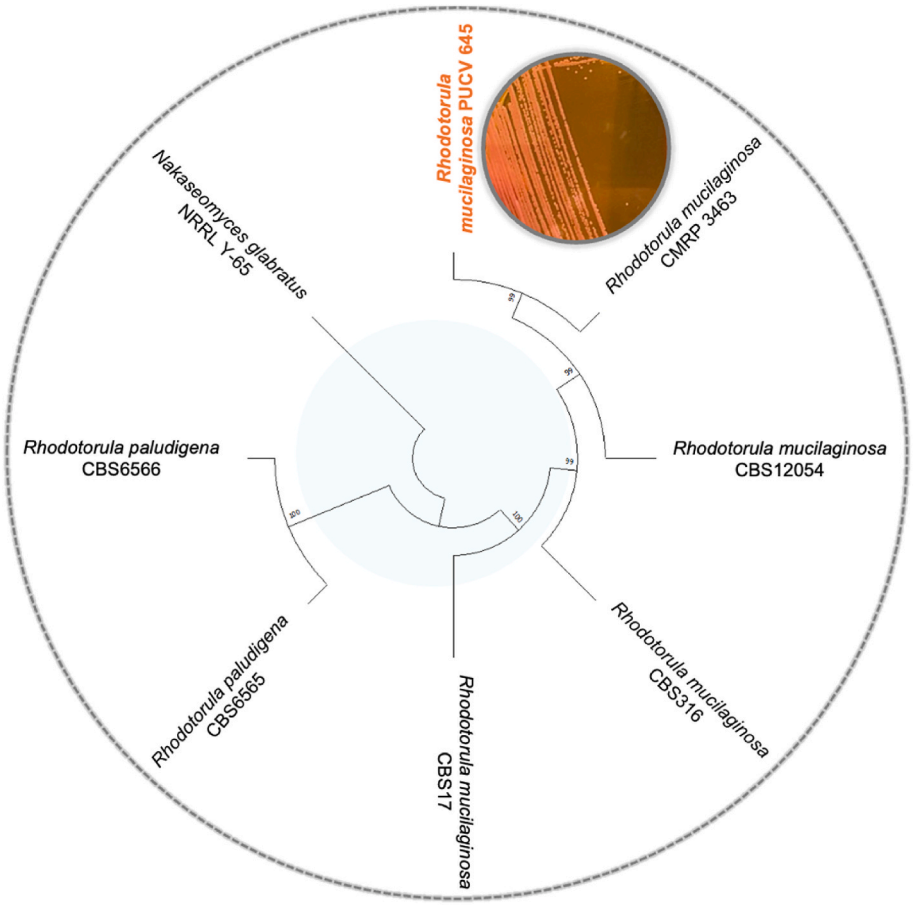

Fig. 2. The phylogenetic tree of *Rhodotorula mucilaginosa* isolate PUCV 645 was constructed using the maximum parsimony method with the TBR algorithm. The analysis included eight nucleotide sequences with 1000 bootstrap replicates, showing the evolutionary relationship of PUCV 645 to closely related species, including *Rhodotorula paludigena*, and it's clear distinction from *Nakaseomyces glabratus*.

difference (ΔE^*_{ab}) was also evaluated to quantify the overall color change. These CIELab values were obtained with an LS173 colorimeter (Shenzhen, China) under standard conditions with D65 as the illuminant and a 10° standard observer. The colorimeter was calibrated using a white plate supplied by the manufacturer before each measurement. The color stability of TRE was evaluated using the CIELab color space system by measuring the total color difference (ΔE^*_{ab}) between initial and final color values after exposure to varying temperatures. Extract samples were subjected to three different temperature treatments (e.g., 50 °C, 70 °C, and 90 °C) for a fixed duration, with color measurements taken at regular intervals.

2.11. Application #1: colored homemade pasta enriched with torulene-rich extracts

In a 250 mL beaker, 80 g of wheat flour, one egg, and 10 mL of colored sunflower oil enriched with TRE (approximately 1.75 μg/g) were combined and thoroughly mixed until a uniform dough formed. The dough was shaped into a ball, wrapped in plastic film, and allowed to rest at room temperature at 25 °C for 30 min. After resting, the dough was divided into manageable portions and rolled out using a Doral pasta maker (Macul, Santiago, Chile) to achieve a thickness of approximately 1–2 mm. The rolled dough was then cut into spaghetti shapes (about 2 mm wide), lightly dusted with flour to prevent sticking, and cooked in boiling salted water for approximately 5 min. A control batch of fresh pasta without TRE was prepared using the same method for comparative analysis. The color of dried and cooked pasta was quantified using a previously described colorimetric method (section 2.10). For color assessment, 10 pieces of pasta were placed in identical positions on a Petri dish. To evaluate the color of cooked pasta, samples were boiled separately for 5 min, dried on a paper towel, and cooled to room temperature (25 °C) before analysis.

2.12. Application #2: colored homemade vegan mayonnaise with torulene-rich extracts and residual aquafaba

Residual aquafaba was obtained from IANSA Agro (Santiago, Chile). Approximately 45 mL of liquid aquafaba was combined with 30 mL of lemon juice, and the mixture was blended using a hand blender for 1 min until a stable emulsion was formed. Then, 118 mL of sunflower oil, enriched with TRE 1 (approximately 1.75 μg/g) and TRE 2 (approximately 3.50 μg/g), was gradually added to the aquafaba-lemon juice mixture while blending at low speed to ensure a smooth and stable emulsion. The mixture was not seasoned with common additives (e.g., salt, vinegar) to avoid interference with the color analysis. A control batch of vegan aquafaba mayonnaise (AM) was prepared using the same procedure but without adding TRE. The color of both experimental and control mayonnaises was assessed, and their stability was evaluated by observing any separation of oil and water phases over 48 h at 4 °C. Color measurements were taken using a colorimeter, following the method previously described for pasta color analysis.

2.13. Statistical evaluation

The results were expressed as mean ± standard error of the mean (SD). Differences among treatments under varying storage conditions

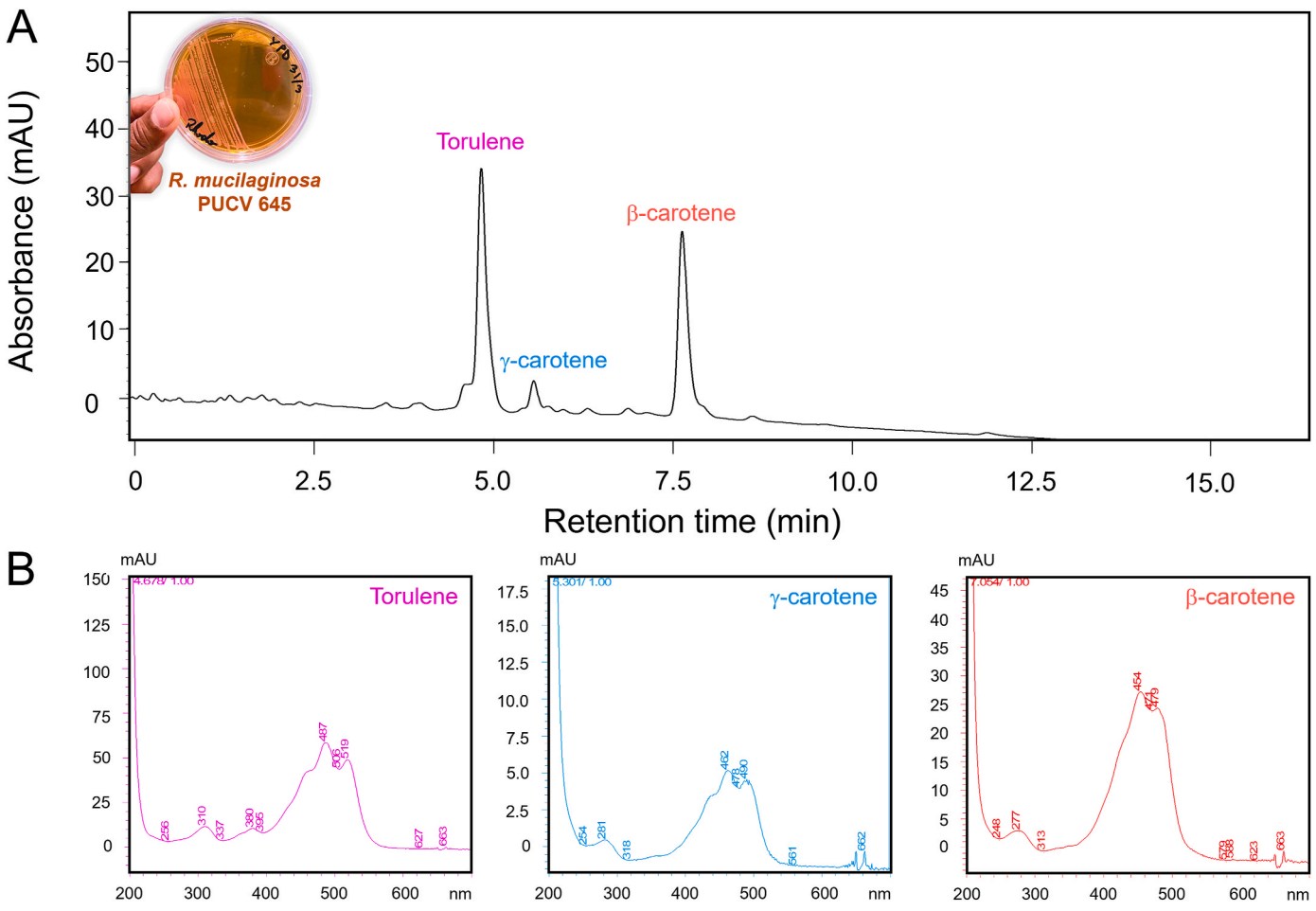

Fig. 3. A- HPLC carotenoid profile (450 nm) and **B-** UV/Vis spectra of major carotenoids (torulene, β-carotene and γ-carotene) from *R. mucilaginosa* PUCV 645 biomass extract.

were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Statistical significance was considered at $p < 0.05$. All analyses were performed using GraphPad Prism version 9 (GraphPad Software, San Diego, CA, USA).

3. Results and discussion

3.1. Identification of R. mucilaginosa isolate PUCV 645

The strain PUCV 645 was isolated from tomato plants collected in a polyethylene greenhouse owned by the Escuela de Agronomía, PUCV (Quillota, Valparaíso region, Chile). Initial streaking on 523 culture plates revealed orange to pink colonies with a globose morphology and defined edges, typical of yeast species (Fig. 2). Furthermore, biochemical characterization, including Gram staining (showing oval, Gram-positive cells), cytochrome oxidase (positive), and catalase activity (positive), provided results consistent with traits reported for members of the genus *Rhodotorula*.

Based on the analysis of ITS region of the rDNA gene, the molecular identification confirmed the biochemical analyses. Sequence alignment and comparison revealed a 98.62 % identity with *Rhodotorula mucilaginosa* (Accession Number: MN638750), confirming the species-level identification. The total sequence length analyzed was 579 base pairs (bp). To confirm the phylogenetic placement of the isolate, a Maximum Parsimony analysis was performed using the MEGA X software. The phylogenetic tree was constructed with the Tree-Bisection-Regrafting (TBR) algorithm and included eight nucleotide sequences from publicly available and well-identified isolates. A total of 636 nucleotide sites were analyzed, of which 569 were conserved. The resulting consensus tree was inferred from 1000 bootstrap replicates, yielding a consistency index of 0.980769, a retention index of 0.980000, and a composite index of 0.974787 (0.961154) for all sites. The phylogenetic tree includes isolates from GenBank obtained from this study and placed isolate PUCV 645 within the *Rhodotorula mucilaginosa* clade, showing a close evolutionary relationship with *Rhodotorula paludigena* while being distinctly separated from other related genera such as *Nakaseomyces glabratus* (Fig. 2).

3.2. Carotenoid identification

The main carotenoids identified in *R. mucilaginosa* PUCV 645 biomass extract in this study were torulene (*m/z* 534), β-carotene (*m/z* 536) and γ-carotene (*m/z* 536), detected in the following corresponding relative percentage 51.3 %, 44.2 % and 4.5 % (Fig. 3).

As depicted in Fig. 3, no torularhodin was detected. It is well known that carotenoid production strictly relies on the yeast species or strains and is also affected by the cultivation parameters used for their bioproduction (Li et al., 2022). Previous literature reported that the yield of torulene could be increased during cultivation in media supplemented with peptone (Kot et al., 2018). This was in accordance with the results obtained in this study, where the carotenoid detected in highest relative percentage, after yeast cultivation in YPD medium was represented by torulene; therefore, the absence of torularhodin in the extract from strain 'PUCV 645' was reported in this study, was probably due to the differences in experimental conditions. In a recent study in which *Rhodotorula glutinis* P4M422 was grown on goat cheese whey, β-carotene,

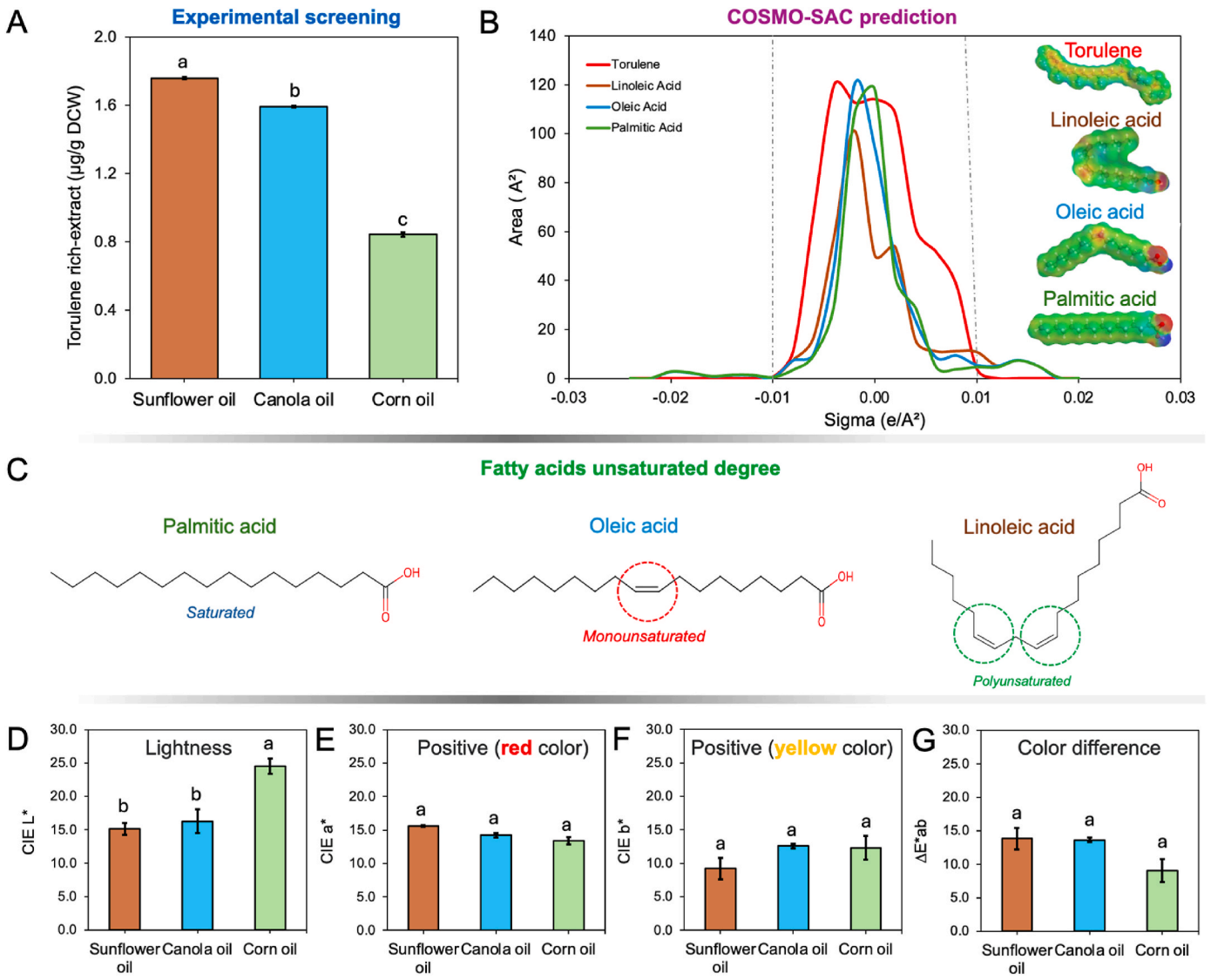

Fig. 4. **A-** Torulene-rich extract concentrations (µg/g) recovered using different edible oils (sunflower oil, canola oil, and corn oil). **B-** Sigma-profile and **C-** charges distribution of torulene and of the major fatty acid in each oil used for used in this work generated by COSMO-SAC. **E- H-** CIELAB color space analysis of torulene-enriched oils: L^* (lightness), a^* (red-green component), b^* (yellow-blue component), and ΔE^*ab (total color difference). Bars represent mean ± standard deviation (n = 3). Different letters above the bars indicate statistically significant differences between groups, according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$).

γ-carotene and torulene, but not torularhodin, were also obtained as major carotenoids (Mata-Gómez et al., 2023). It has been reported that both torulene and torularhodin production are correlated to a protective effect against membrane impairment by activated oxygen molecules, highlighting the concept of their antioxidant role in the cells and their production as a response to high dissolved oxygen percentage. Moreover, interestingly, both torulene and torularhodin have been reported to have anti-microbial and anti-cancerous properties (Kot et al., 2018).

3.3. *Understanding the torulene-rich extracts recovery using edible oils*

The strategy to use edible oils was selected as extractant agents due to their hydrophobic nature (Al-Maari et al., 2024; Caicedo-Paz et al., 2024), which facilitates the solubilization of hydrophobic compounds such as torulene (Mussagy et al., 2020). Additionally, the use of edible oils (i.e., canola, sunflower and corn oil) allows the direct application of torulene-enriched oils in food-related applications, aligning with the requirements of the food industry for natural and safe ingredients (Knorr, 2024). In this work, the recovery of torulene-rich extracts from new PUCV 645 using edible oils as extractant solvents demonstrated significant differences in extraction yields depending on the molecular structure of the main fatty acid in each oil (Fig. 4). The results depicted in Fig. 4A revealed that sunflower oil achieved the highest torulene-rich extract concentration (1.75 µg/g), followed by canola oil (1.59 µg/g) and corn oil (0.84 µg/g). The high recovery yields torulene-rich extract using sunflower oil is attributed to its specific composition *viz.*, linoleic acid, a polyunsaturated fat (Caicedo-Paz et al., 2024), which can enhance the solubilization of hydrophobic compounds like torulene. Canola oil mainly composed 50 %–65 % oleic acid, a monounsaturated fat (Ahmad et al., 2024), also demonstrated relatively high extraction efficiency; although the concentration of torulene obtained with this oil was slightly lower than that observed with sunflower oil, the difference remains minimal, suggesting that both oils can be considered efficient extraction solvents. On the other hand, corn oil yielded the lowest concentration of torulene, which may be explained by its composition, *viz.*, 80 % of palmitic acid (Huda & Monono, 2024) with lower affinity for torulene.

The differences in the extraction yields result from the oil polarity

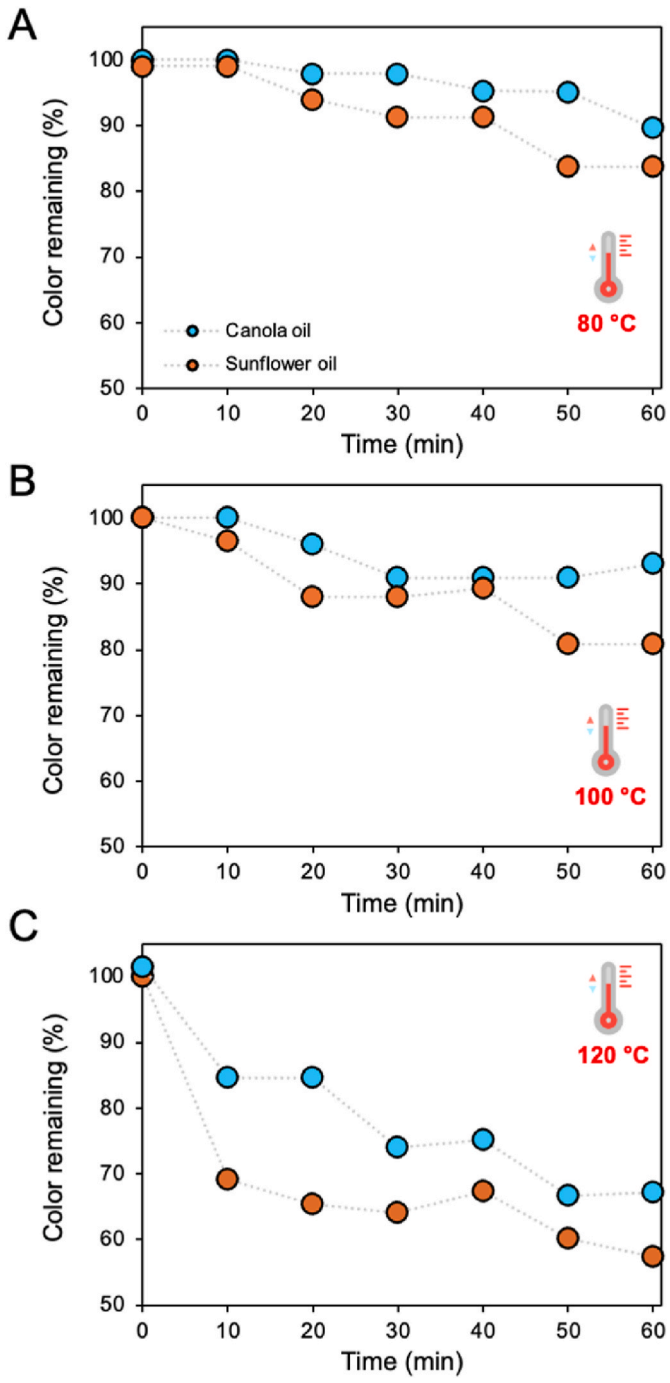

Fig. 5. Color stability of torulene-rich extracts in edible oils (sunflower and canola) at different temperatures (**A**- 80 °C, **B**- 100 °C, and **C**- 120 °C) over time; Data are presented as mean values (n = 3).

and fatty acid composition. In Fig. 4B,C and **D** it is possible to see the sigma-profile (σ-profile), charges distribution and chemical structure of the torulene and of the major fatty acid in each oil under evaluation: linoleic acid (sunflower), oleic acid (canola), and palmitic acid (corn). The extraction yields increase with the unsaturated degree of the fatty acids, following the order: linoleic acid (C18:2) > oleic acid (C18:1) > palmitic acid (C16:0). Consequently, the fatty acids polarity reduces in the same order.

For a deeper discussion, the σ-profile (Fig. 4B) can be taken into consideration. The σ-profile is a graphical representation of the induced charge distribution around a specific molecule (Goltz et al., 2021), *i.e.*, the polar and nonpolar regions of the molecule. The fatty acids under evaluation present a predominant nonpolar region (area between 0.01 and -0.01 e/A^2) and small polar regions ($0.01 < e/A^2 < -0.01$). Torulene, in turn, is an entirely neutral molecule, which means that its extraction will occur through an intermolecular interaction (i.e., Wan der Waals force and π-π interactions) with the non-polar region of the fatty acid. However, the higher extraction capacity was obtained with the major fatty acid with the lower nonpolar region. It suggests that double bonds play an important, critical, and essential role in this kind of extraction. Oils rich in unsaturated fatty acids have a less compact and more fluid structure and, consequently, are less viscous, benefiting the solute-solvent contact and the diffusivity during the extraction (Fig. 4C). In general, our results suggest that both the carbon chain of the fatty acids—and consequently, the triacylglycerols in the oil—and the presence of double bonds contribute to torulene recovery. The first issue is responsible for forming intermolecular interaction with the solute (torulene), while the second one makes this interaction easier.

To further explore the potential of oil-torulene-rich extracts as natural food colorants, a comprehensive colorimetric analysis was conducted using the CIELAB color space. This analysis provided insights into the visual properties of sunflower, canola, and corn oils, specifically lightness (L*), red-green (a*), and yellow-blue (b*) components, along with the total color difference (ΔE*ab). Statistical analysis (ANOVA with Tukey's HSD post-hoc test) revealed that sunflower oil's L* value was significantly lower ($p < 0.05$) than that of corn oil. The average a* (15.59) and b* (9.22) values were not significantly different ($p > 0.05$) from canola or corn oil. The average color difference (ΔE*ab) was 13.83. Canola oil showed an average L* value (16.27). Its L* value was also significantly lower ($p < 0.05$) than that of corn oil. Statistical analysis revealed no significant differences ($p > 0.05$) in a* (14.23) and b* (12.60) values between canola oil and sunflower or corn oil. The average ΔE*ab was 13.63. Corn oil yielded the highest average L* value (24.49). This value was significantly higher ($p < 0.05$) than those of sunflower and canola oils. There were no significant differences ($p > 0.05$) in a* (13.39) and b* (12.31) values compared to sunflower and canola oils. The average overall color difference (ΔE*ab) was 9.03. Given that ΔE*ab values above 3 CIELAB units are noticeable by humans with normal vision (Fernández-Vázquez et al., 2013), the significant differences ($p < 0.05$) in L* values, coupled with the higher ΔE*ab values for sunflower and canola oils compared to corn oil, suggest that the color differences are visually distinct.

3.4. Color stability of torulene-rich extracts in edible oils

Torulene-rich extracts, due to their ability to provide color and potential health benefits (Jin et al., 2024), are being considered for producing colored pasta that will undergo cooking at high temperatures. Given that color stability is a critical factor in the acceptance of the final product, evaluating the effects of elevated temperatures on extracts is essential. For that, the color stability of torulene-rich extracts was evaluated in edible oils with the highest recovery yields—sunflower and canola—at three different temperatures (80 °C, 100 °C, and 120 °C) to assess the thermal degradation behavior of the pigment over time. The ΔE^*_{ab} values were employed as indicators of the total color difference (Fig. 5), highlighting the impact of temperature on carotenoid stability, including torulene. As illustrated in Fig. 5A, at 80 °C, the degradation of TRE was minimal in both oils. Notably, canola oil demonstrated higher color stability throughout the 60-min analysis period, with values remaining nearly constant (approximately 93–100 %). This indicates that TRE exhibited significant resistance to thermal effects at this moderate temperature. In contrast, sunflower oil experienced a more pronounced color loss, with values decreasing to approximately 80.74 % by the end of the heating period. This differential stability suggests that canola oil may offer a more favorable environment for preserving carotenoid integrity under moderate thermal conditions.

As depicted in Fig. 5B, increasing the temperature to 100 °C resulted in a noticeable decline in color stability for both oils over time. Canola

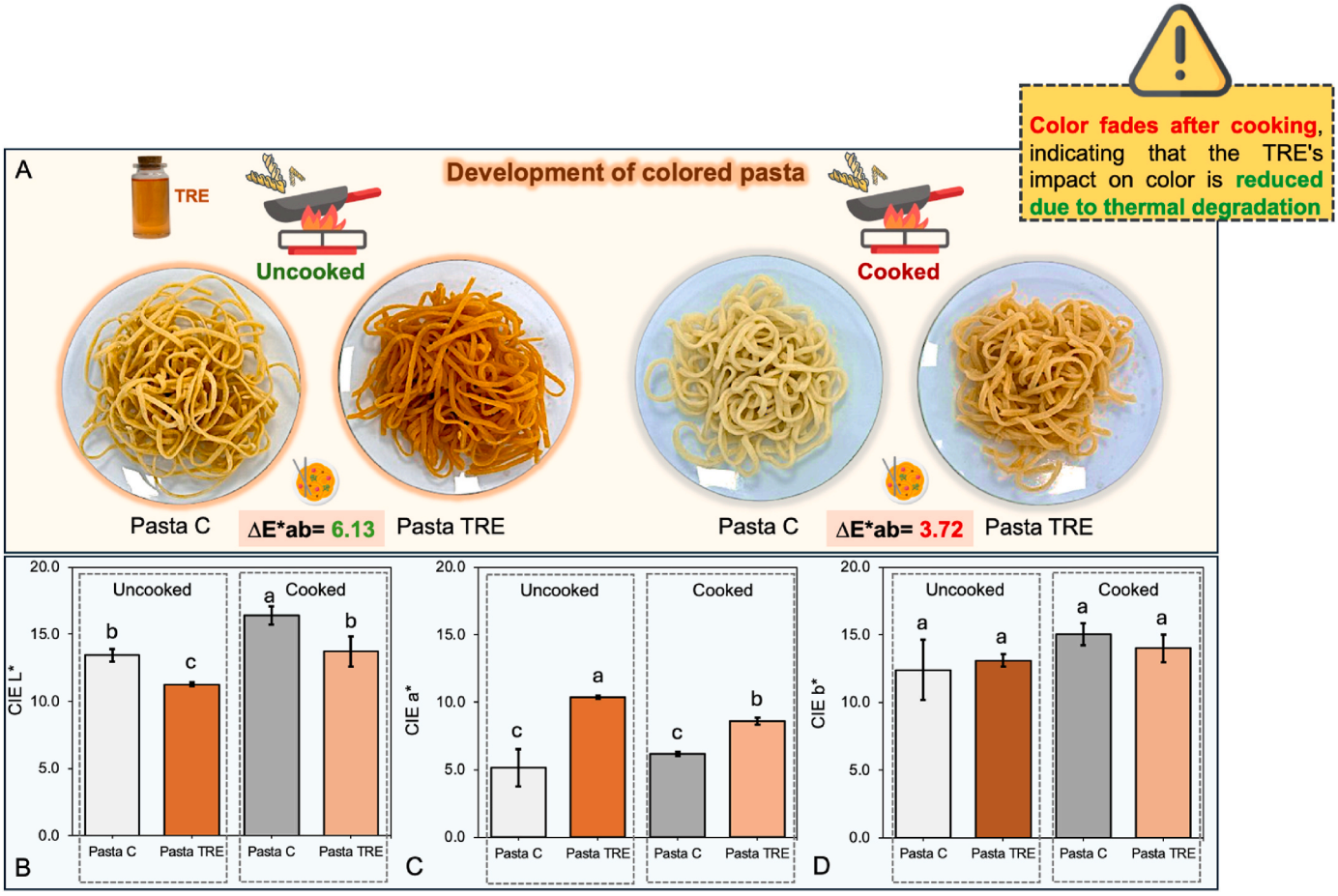

Fig. 6. A- Uncooked (left) and cooked (right) pasta with TRE. **B-D**- CIELAB color space analysis of pasta: L* (lightness), a* (red-green component), and b* (yellow-blue component). Note that, the uncooked pasta shows a vibrant color with higher redness and slight yellowing, but the color significantly fades after cooking, indicating that the TRE's impact on color is reduced due to thermal degradation during the cooking process; Bars represent mean ± standard deviation (n = 3). Different letters above the bars indicate statistically significant differences between groups, according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$).

oil continued to exhibit better stability, retaining approximately 89.62 % of its color after 60 min, compared to sunflower oil, which dropped to 83.78 %. These findings suggest that canola oil may provide a more protective environment for torulene-oleic acid. It can also be related to the previous discussion concerning the unsaturation presence, i.e., canola oil is rich in oleic acid, which presents only one double bond, consequently presenting a more compact structure than sunflower, reducing the TRE mobility in the oil and improving this stability.

These findings suggest that canola oil may provide a more protective environment for torulene-oleic acid interaction, thus contributing to a reduced rate of oxidative degradation, thereby preserving the color and stability more effectively than sunflower oil. At the elevated temperature of 120 °C, the color stability of TRE was significantly compromised in both oils, indicating increased degradation rates. Sunflower oil exhibited rapid color loss, decreasing to approximately 60 % after 60 min, while canola oil retained around 67.13 % by the end of the heating period. This marked reduction in stability at higher temperatures underscores the vulnerability of carotenoids to thermal degradation and suggests that both oils have limitations when subjected to extreme heat (Mayumi Ueda et al., 2023; Syawalluddin et al., 2024).

3.5. Use of torulene-rich extracts in food manufacturing

3.5.1. Application #1: torulene-rich extracts on the color of pasta

From what it was commented earlier, TRE can be used as an innovative colorant agent with possible health-promoting and preservative actions. The influence of TRE on pasta color was evaluated by analyzing the colorimetric parameters and the total color difference in both uncooked and cooked pasta samples (Fig. 6).

The addition of TRE significantly altered the color profile of uncooked pasta (Fig. 6A, *left*). Compared to the control sample ($L^* = 13.45$, $a^* = 5.14$, $b^* = 12.41$), the TRE-enriched pasta exhibited reduced lightness ($L^* = 11.26$) and notably higher redness ($a^* = 10.36$), accompanied by a slight increase in yellowness ($b^* = 13.10$) (Fig. 6B–D). These changes resulted in a substantial total color difference ($\Delta E^*ab = 6.13$), therefore, the significant impact of TRE on the product's visual appearance was noticeable (Fig. 6A, *left*). After cooking, the TRE-enriched pasta retained a distinct color profile compared to the control. Lightness decreased from $L^* = 16.39$ (control) to $L^*13.71$, redness increased from $a^* = 6.16$ to $a^* = 8.58$, and yellowness slightly reduced from $b^* = 15.05$ to $b^* = 14.01$. However, the calculated total color difference ($\Delta E^*_{ab} = 3.72$) was reduced than in the uncooked samples, highlighting the stability of the TRE pigments under thermal processing (Fig. 6A, *right*).

It is noticeable that the cooking process notably affected the color in pasta enriched with TRE. These changes can be attributable to a great extent to the partial degradation of TRE during thermal processing, as well as structural changes in the pasta matrix, such as starch gelatinization, which may affect light scattering and color perception (Suo et al., 2024; Toultchinski & Vilgis, 2024). Despite these changes, the TRE-enriched pasta still retained a visually distinct and appealing coloration after cooking, underscoring the potential of TRE as a natural colorant suitable for processed foods.

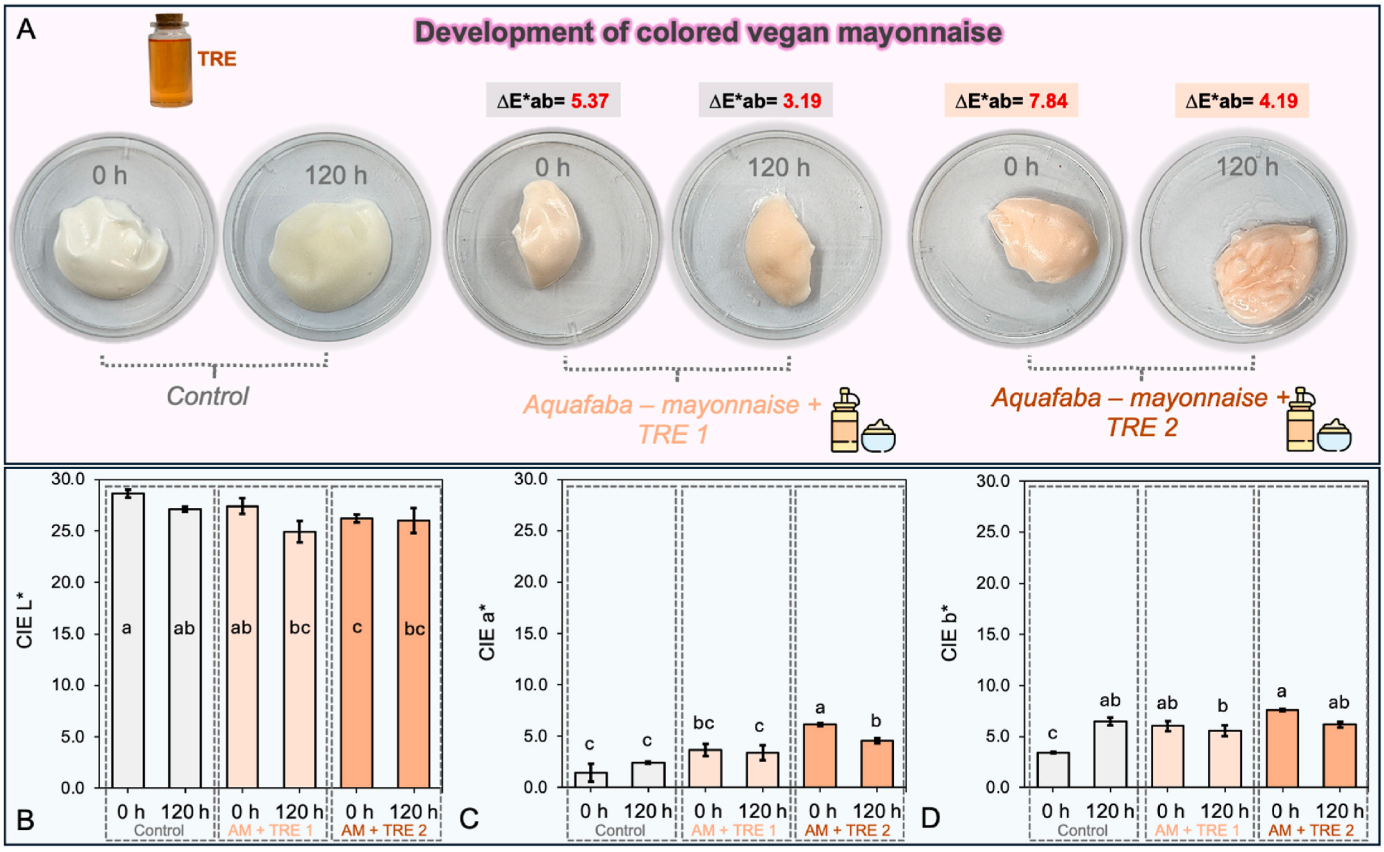

Fig. 7. A- Colorimetric parameters (L^*, a^*, b^*) and total color difference (ΔE^*_{ab}) for control aquafaba mayonnaise (AM) and formulations enriched with TRE (AM + TRE 1 and AM + TRE 2) at 0 h and after 120 h of storage; Bars represent mean ± standard deviation (n = 3). Different letters above the bars indicate statistically significant differences between groups, according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$).

3.5.2. Application #2: torulene-rich extracts combined with residual aquafaba as potential egg replacer for vegan mayonnaise

As observed in the previous section (*application #1*), thermal processes, can significantly alter the color and properties of food products, including those enriched with TRE. This limitation calls for exploring alternative applications where 'heat' is not required. One such application is using TRE in combination with residual aquafaba (a potential egg replacer) for vegan mayonnaise. By combining TRE with aquafaba, this study aims to develop a plant-based mayonnaise that not only mimics the texture and functionality of traditional mayonnaise but also enhances its color and nutritional profile without the need for thermal processing (Fig. 7).

As depicted in Fig. 7, the colorimetric parameters (L^*, a^*, b^*) and total color difference (ΔE^*_{ab}) for control AM and formulations enriched with TRE (AM + TRE 1 and AM + TRE 2) at 0 h and after 120 h of storage, revealed that initially, both AM-TRE-enriched samples demonstrated significant color changes compared to the control. For AM + TRE 1, a notable increase in redness ($a^* = 3.7$ *vs.* 1.46 for the control) and yellowness ($b^* = 6.04$ vs. 3.44 for the control) was observed, resulting in a total color difference of $\Delta E^*_{ab} = 5.37$. Similarly, AM + TRE 2 exhibited an even more significant color enhancement, with the highest redness ($a^* = 6.15$) and yellowness ($b^* = 7.61$), contributing to an $\Delta E^*_{ab} = 7.84$, which indicates a striking visual distinction Fig. 7A. Over the 120-h storage period, a slight reduction in color parameters was observed across all samples. The redness and yellowness of AM + TRE 1 decreased to $a^* = 3.39$ and $b^* = 5.55$, respectively, leading to a reduced $\Delta E^*_{ab} = 3.19$. For AM + TRE 2, although the color remained more stable, a slight decrease in a^* (4.6) and b^* (6.18) resulted in a $\Delta E^*_{ab} = 4.19$. These results highlight the potential of TRE to enhance the visual appeal of vegan AM while maintaining acceptable stability during storage.

In fact, unlike in the colored pasta application, where thermal processes led to partial degradation of TRE pigments, the cold-processing nature of mayonnaise production preserves the color integrity of TRE, making it a promising candidate for enhancing plant-based formulations. Furthermore, incorporating TRE improves the aesthetic properties and could contribute to the nutritional and antioxidant profile of the final vegan AM product.

4. Conclusions

This study demonstrates the significant potential of newly isolated *R. mucilaginosa* isolate PUCV 645 as a source of carotenes, particularly torulene. The yeast was successfully identified and characterized the isolate, confirming its identity through molecular techniques and phylogenetic analysis. The main carotenoids detected in the biomass extract were torulene (51.3 %), γ-carotene (4.5 %), and β-carotene (44.2 %), highlighting torulene as the predominant compound. Furthermore, TRE was effectively recovered using various edible oils, with sunflower oil yielding the highest concentration of TRE at 1.75 μg/g. The interesting applications of TRE in food products were demonstrated through the development of colored homemade pasta and vegan mayonnaise. The pasta enriched with TRE exhibited a distinct color profile, indicating the successful incorporation of the natural colorant. The vegan mayonnaise also benefited from adding TRE, enhancing its visual appeal without compromising stability. As observed, this study not only confirms the potential of new *R. mucilaginosa* isolate PUCV 645 as a natural source of carotenoids but also demonstrates a possible practical application in food products that align with consumer demands for natural ingredients. Further studies are in progress in order to evaluate the

antioxidant activity and how the TRE supplementation can affect the shelf life of the food products proposed.

CRediT authorship contribution statement

Cassamo U. Mussagy: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Debora Figueroa:** Investigation. **Ximena Besoain:** Formal analysis. **Natalia Riquelme:** Investigation. **Aldo Salinas:** Investigation. **Daniele Giuffrida:** Writing – original draft. **Alessia Tropea:** Investigation. **Francesca Rigano:** Investigation. **Luigi Mondello:** Resources. **Fabiane O. Farias:** Investigation. **Antonio J. Meléndez-Martínez:** Writing – review & editing. **Angie V. Caicedo Paz:** Writing – original draft, Methodology, Investigation.

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Declaration of competing interest

There are no conflicts to declare.

Data availability

Data will be made available on request.

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