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Optimization of Total Carotenoid Production by *Rhodotorula mucilaginosa* from Artichoke Agroindustrial Waste Using Response Surface Methodology

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The carotenoids have vast medical, industrial, dietary, and pharmaceutical importance due to their provitamin A precursor, immunomodulator, antioxidant and photoprotective activity. The purpose of the research was to optimize the production of carotenoids using *Rhodotorula mucilaginosa* from artichoke agroindustrial waste as a low-cost substrate. The artichokes bracts waste was bleached by sodium hypochlorite (NaClO 2%) and was characterized through whiteness index and FTIR. The bleached artichoke waste (BABW) used in the fermentation went through acid hydrolysis, applying 8% of the bleached artichokes residue and sulfuric acid (2.5%) for 1 h at 90°C, obtaining a greater reduced sugars content at 3.1 g/L. *Rhodotorula mucilaginosa* was isolated and molecularly identified. The production of carotenoids from a culture media based on hydrolyzed BABW, peptone (0.5%), yeast extract (0.1%) and sodium chloride (0.5%) was evaluated at different conditions of pH (5–8) and agitation speed (80–160 rpm) applying the surface response methodology by a rotational central compound design. The best carotenoids performance obtained had 2968.95 µg/L VVC and 1228.53 µg/g TFC at pH 5, 120 rpm and 30°C for 72 h. The chemical

characterization of the extracted carotenoids was confirmed by UV-VIS and Raman spectroscopy methods. The results suggest that *Rhodotorula mucilaginosa* is capable of producing carotenoids from artichoke waste fermentation, providing a low-cost and sustainable alternative route for use in the global market.

Keywords: waste, artichoke, carotenoids, *Rhodotorula*, biotechnology.

Introduction

Carotenoids are natural liposoluble pigments of highly conjugated tetraterpenoid structure and light reflexive properties, showing a diversity of colors such as red, orange, yellow, or purple (Zhao et al., 2019). The total carotenoids are found as carotenes (α, β, γ-carotene and lycopene) and xanthophylls (lutein, zeaxanthin and others) (Maoka, 2020); similarly, they exhibit different bioactive properties: antioxidants, provitamin A precursors, immunomodulators and photo protectors (Amengual, 2019). Carotenoids are mainly extracted from plant sources which require strict and unsustainable chemical processes due to the use of toxic petrochemical solvents (Kultys and Kurek, 2022). Therefore, obtaining carotenoids from microbial sources such as filamentous fungi, bacteria, microalgae and yeast provides an affordable and sustainable alternative, since it allows greater productivity in a short time, absence of seasonal restrictions, and it is non-toxic, facilitated a controlled fermentative process with generation of biodegradable by-products (Ram et al., 2020). The yeasts stand out as producers due to their fast growth and carotenoid production, including genera as *Rhodotorula, Sporobolomyces* and *Xanthophylomyces* (Menegazzi et al., 2020).

The genus *Rhodotorula* genus is the most studied because of its aerobic metabolism and simple nutrition based on carbon and nitrogen, providing its substrate availability, low cost, and elevated cell growth (Liu et al., 2021). To improve the fermentation process, new environment-friendly paths are being acquired with the use of cost/effective substrates including vegetable waste (Kaur et al., 2019). For example, Dias-Moreira et al. (2021) reached an efficiency of 361.3 and 296.6 μg/g of total carotenoids from coffee pulp and skin, respectively. In addition, Sharma and Ghoshal (2020) obtained a maximum content of carotenoids (717.82 mg/g) from an acid extract of onion skin and mung bean at 25° and pH 6 at 110 rpm. In the same way, Cipolatti et al. (2019) achieved to use sugarcane molasses and corn-macerated liquor to obtain an efficiency of 224.8 μg/L volumetric carotenoids using *Rhodotorula mucilaginosa.*

The agricultural sector is considered an important livelihood activity for the world economy, through the supply of fresh food for consumption and raw materials to the food industry. However, it is estimated that 1300 million metric tons of food are wasted annually, the vegetables, tubers and fruits being the ones with the greatest quantity (Yafetto, 2022). In this way, agro-industrial waste has become an alternative raw material for industrial use due to its wide availability (Ravindran et al., 2018). The agroindustrial waste can be highlighted for the fermentative production of carotenoids, as Otero et al. (2019) got 820 μg/L of carotenoids by *Rhodotorula mucilaginosa* from parboiled rice water. Likewise, Torres-Alvarez et al. (2019) got 317 μg/g of carotenoids by *Rhodotorula mucilaginosa* from banana peel extract, and Sinha et al. (2019) achieved to obtain 20 mg/L by *Rhodotorula toruloides* at 120 h from paddy straw hydrolysate.

Peru is considered the first exporter of fresh vegetables in South America and the eighteenth globally, with 8.34% of artichoke's production generating an export of 10 000 annual tons (Chavez, 2021). In artichoke processing, the resulting waste is 80–85% of the total vegetal biomass, such as bracts which are the external parts of flowers (Monge et al., 2019). The waste of artichoke bracts has a profitable structural composition, with up to 43% of cellulosic content and 10.4 g/100 g reducing sugars, as well as fructose polysaccharides at 15.9 g/100 g of dry weight (Zeaiter et al., 2019). However, little has been used for the production of carotenoids by fermentation.

In this way, the use of artichoke bracts waste represents a sustainable alternative for the collection of carotenoids by biotechnological paths. The main purpose of this investigation was focused on assessing the use of artichoke bracts coming from agroindustrial waste for the production of total carotenoids from a culture of *Rhodotorula mucilaginosa*.

Methods

Isolation and characterization of *Rhodotorula mucilaginosa*

Different agricultural soil samples were collected (1 kg) from the Center of Plan Genetics at Universidad Nacional de Trujillo, Perú (8°06'46.2"S, 79°02'18.7"W). A suspension of 10 g of soil in 100 mL of sterile saline solution (0.85% NaCl) was prepared, homogenized and left to rest at room temperature for 15 minutes. Serial dilutions were gotten from the supernatant, and aliquots of 100 µL were inoculated over the surface of a Petri dish with agar Sabouraud (glucose 4%, yeast extract 0.1%, peptone 0.5% and sodium chloride 0.5%; pH 6), then it was incubated at 30ºC for 48 h. The signature *Rhodotorula*-colored colony was selected and its purity was determined by testing its macroscopical and microscopical morphological features (El-Ziney et al., 2018).

The isolated yeast culture was identified through molecular analysis with the use of a Polymerase Chain Reaction (PCR) of the ITS1 e ITS2 (subunit 5.8S rRNA), according to the methodology of Baldera et al. (2022). The DNA was extracted from a highly pure 48 h culture (Nucleic acids extraction and purification kit, innuPREP DNA Kit, Analytik Jena). The amplicon was obtained through the ITS-1 and ITS-2 universal primers, immediately after the amplified rDNA was characterized in a 1.5% agarose gel. The purified PCR product was sequenced (Macrogen, USA) and aligned with sequences through BLAST (National Center for Biotechnology Information, NCBI). A phylogenetic tree was elaborated with the rDNA sequences using MEGA-X software.

Collecting and setting up of agroindustrial waste

The artichoke bracts waste (ABW) was collected from agro-export businesses in La Libertad region, Peru. The ABW were washed and dried at 60°C for 18 h on a stove (MMM, ECOCELL 111). After that, they were ground and sifted at a pore size of 600 µm (Sadh et al., 2018).

The 2% (a/w) of sifted ABW was then bleached (sodium chloride 2%) for 1 h at room temperature (Widiarto et al., 2017). Subsequently, the bleached ABW was vacuum filtered and then washed with distilled water in order to remove chlorine waste. The bleached artichoke bracts waste (BABW) was spread out on a plastic tray and dried at 60°C for 18 h in a stove (MEMMERT, UF260 PLUS). Then, it was pulverized and kept in polyethylene bags at room temperature until its use (20 ± 2°C).

Characterization of the agroindustrial waste

The chemical features were determined through infrared spectroscopy from a sample of 0.5 g BABW and ABW, using a Thermo Nicolet IS50 (USA) device through attenuated total reflectance (ATR) at 4000–500 cm^{-1} (Trilokesh and Uppuluri, 2019). The colors of the reflecting surface of the residue were measured using a spectrophotometer with a color measuring system (Konica Minolta CM-5, Tokio, Japón). The L*, a* and b* values were registered from the waste sample (Aridi et al., 2021).

$$WI\ (\%) = 100 - [(100 - L^*)^2 + (a^*)^2 + (b^*)^2]^{1/2} \quad (1)$$

Fermentable sugars extraction from BABW

The extraction was carried out according to the acid hydrolysis method described by Prasertsung et al. (2019). The effect of sulfuric acid was assessed (0.5%, 1% and 2.5%) over different proportions of RBAB (1%, 2%, 4% and 8%) at 80°C for 60 minutes. The hydrolyzed BABW was separated through filtration and neutralized with NaOH (2N). The fermentable sugars were determined by the 3,5-dinitro salicylic acid (DNS) method (Achara and Pannarai, 2016).

Fermentative production of carotenoids

The effect of pH (5–8) and stirring speed (80–120 rpm) were assessed in the carotenoids production by a Central Composite Rotational Design (CRD) (*Table 1*). The experiment was done in 150 mL flasks with 30 mL of the medium from the hydrolyzed BABW supplied with 0.5% peptone, 0.1% yeast extract, and 0.5% sodium chloride. The initial cell content of the yeast inoculation was 2.6 ± 0.1 at OD_{600}, previously cultured. The pilot testing was incubated at 30ºC for 72 hours in agitation according to the experimental design, using a shaking

Table 1. *Used values at the Central Composite Rotational Design for two independent variables*

Independent variables	Coded levels of variable				
	–1.4	–1	0	1	1.4
pH	5	5.428571	6.5	7.571429	8
Agitation (rpm)	80	91.42857	120	148.5714	160

incubator (LBSI-100A, LABNICS). The yeast biomass was monitored through optical density using a UV-VIS spectrophotometer (UVLINE, 9400).

Total carotenoids extraction and estimate

The carotenoids were extracted with dimethyl sulfoxide (DMSO, Merck) according to the method suggested by Kanzy et al. (2015). The obtained biomass was centrifuged at 6000 rpm for 5 min, the supernatant was removed and washed two times with distilled water. The achieved biomass was resuspended in 20 mL of distilled water and poured into a Petri dish to dry at 45°C for 24 hours on a stove (MMM, ECOCELL 111). 25 mg of dry biomass was weighted and resuspended in 1 mL of DMSO, the mixture was agitated in a vortex for 30 seconds and it was put into Termoblock (VRS, 12621-092) at 55°C for 1 hour, with agitation in a vortex every 15 minutes. Finally, the sample was centrifuged for 5 minutes at 6000 rpm and the pigmented supernatant was recovered for its optical reading at 501 nm through a UV-VIS spectrophotometer (UVILINE, 9400).

The total content of carotenoids was calculated and expressed as Total Fraction of Carotenoids, TFC, (µg/g of dry yeast) and volumetric carotenoids concentration, VCC (µg/L) (Dyaa et al., 2022), according to the next formula:

$$\text{VCC (µg/L)} = \frac{(A)(V)(10^6)}{E_{1cm^{1\%}}(1000)} \quad (2)$$

Where: A – absorbance at 501 nm; V – total carotenoids volume (mL); Extinction coefficient ($E_{1cm^{1\%}(DMSO)}$) = 2040.

Total fraction of carotenoids:

$$\text{TFC (µg/g)} = \frac{CVC\ (\frac{\mu g}{L})}{CBC\ (\frac{g}{L})} \quad (3)$$

$$\text{CBC (Cell Biomass Concentration)} = \frac{Biomass\ dry\ weight\ (g)}{Fermentation\ breeding\ volume\ (L)} \quad (4)$$

Characterization of extracted carotenoids

The maximum absorbance of the extracted carotenoids solution was analyzed using a spectrophotometer (UVILINE, 9400) in the 300–800 nm range. The dry biomass obtained was analyzed through Raman confocal spectroscopy with the use of an Ar laser of 514.5 nm and it was assessed within 800–1800 cm^{-1} spectra with a 2 cm^{-1} (Jehlička et al., 2014).

Statistical analysis

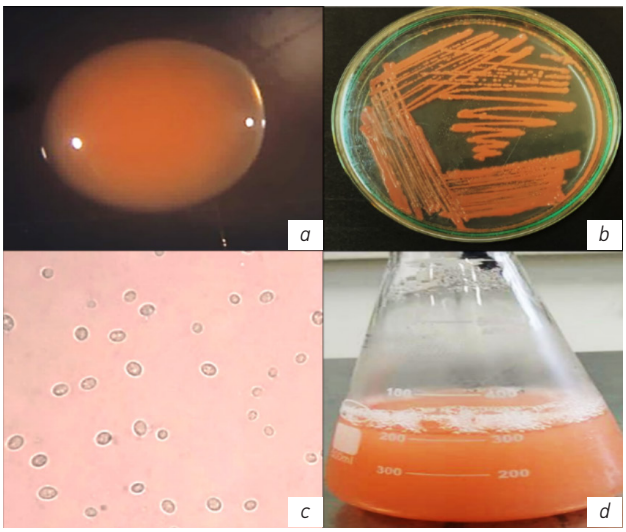
The obtained data were subjected to an analysis of variance (ANOVA). They were calculated as media ± standard deviation, and the resulting surfaces were analyzed using Design Expert 13, with statistical significance $P < 0.05$ (Allahkarami et al., 2021).

Results and Discussion

Isolation and identification of *Rhodotorula mucilaginosa* strain

A total of 10 different morphologies were obtained from microbial isolations, but just a culture showed yeast-like growth with coral pink pigment (designed as TQ21). The isolated TQ21 showed convex colonies of smooth edges with brilliant and coral pink color of mucoid aspect. The cells from TQ21 isolate were verified through microscopy, where blastoconidia and mono-budding ovoid cells (*Fig. 1*). Additionally, the liquid medium turned yellow to red after 48 hours of growth; these features coincide with Hu et al.'s (2022) reports where they isolated and described different morphological similarities of the *Rhodotorula* genus.

Fig. 1. *Isolation and morphological identification of the TQ21 culture: a, b) colonies of the high purity culture of strain TQ21 in Sabouraud agar medium with 48 hours of growth; c) microscopical view of the yeast-like cells at 400X view; and d) cell growth in a yeast-peptone dextrose extract at 48 hours*



The PCR amplification of regions ITS-1 and ITS-2 of gen 5.8S rRNA produced an amplicon of 159 bp; these sequences were compared with the GenBank database (BLAST). The sequence alignment revealed that the isolated strain showed a 99% similarity with *Rhodotorula mucilaginosa* PHYTYM9 (MT559975.1); likewise, the phylogenetic analysis based on the sequence of the region ITS 1-2 of gen 5.8S rRNA placed the TQ21 strain near the *Rhodotorula mucilaginosa* group with a branch length of 0.09 indicating a minor species divergence at the nucleotide level (*Fig. 2*). The isolated yeast was identified as *Rhodotorula mucilaginosa* TQ21 and the sequence noted was posted in the NCBI database with access number OM721682.1 (https://www.ncbi.nlm.nih.gov/nuccore/2195005066).

Characterization and conditioning of the waste

The FTIR waste spectrum without treatment showed a peak of over 3290 cm^{-1} attributed to the O–H stretching vibration contained in its cellulose, hemicellulose, and lignin (Yuli et al., 2020). In the same way, the 2920 cm^{-1} peak was assigned to the C-H cellulose stretching (Zheng et al., 2019). The 1730 cm^{-1} band was assigned to the C=O lignin vibration (Falah et al., 2020). The 1610 cm^{-1} peak corresponded to the O–H vibrations due to the water traces (Mat, 2014). Also, the peaks in 1410–1420 cm^{-1} were associated with the group CH_2, related to cellulose crystallinity; while, 1370 cm^{-1} to the C–H deformation in cellulose and hemicellulose (Akcay and Yalcin, 2021). At the same time, band 1320 cm^{-1} was related to the vibration of the C–H syringyl and guaiacyl lignin ring (Zheng et al., 2015). According to Bolio-López et al. (2016), the peak within 1240–1250 cm^{-1} was assigned to aromatic ester groups (syringyl lignin). The peak 1030 cm^{-1} was attributed to the stretching vibration of CO and OH groups; and 898 cm^{-1} to β-glucoside links (De-Rosa et al., 2010). The presence of lignin in the artichoke waste linked to its cellulose was corroborated, which represents a tipping point for hydrolysis (*Fig. 3*) (Ma et al., 2014).

Sodium hypochlorite (NaClO) was used for the selective removal of lignin since this last mentioned behaves as a recalcitrant and fermentation inhibitor (Morone et al., 2017). After treating the residue with NaClO, a variation in the transmittance percentage was observed in the featuring peaks of lignin at 1720–1730, 1320, and 3290–3330 cm^{-1} (*Fig. 3*), probably because of the decrease in the lignin content (Aridi et al., 2021). This lignin decrease was contrasted through the whiteness

Fig. 2. *The phylogenetic tree was inferred using Neighbor-Joining method with a 1000 number Bootstrap. The evolutional distances were calculated using Tamura 3-parameter based on the nucleotide replacement. Yarrowia lipolytica CBS 6124T (NR_111212.1) was selected as an external group*

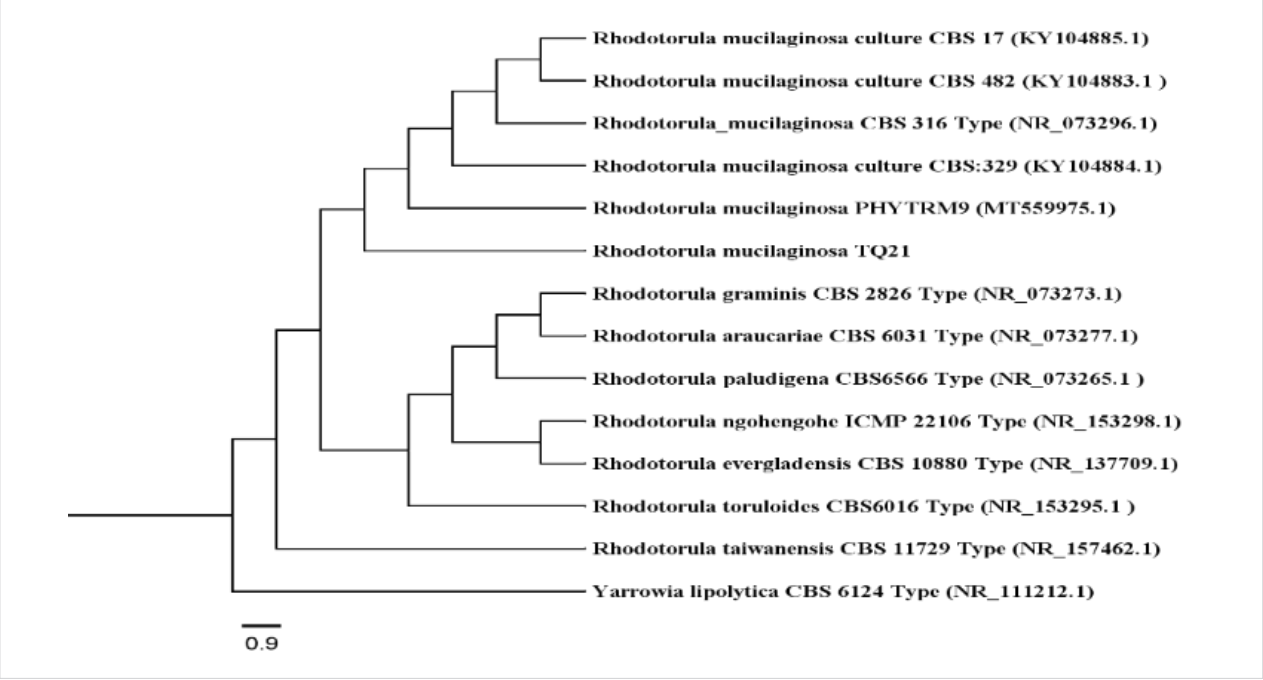
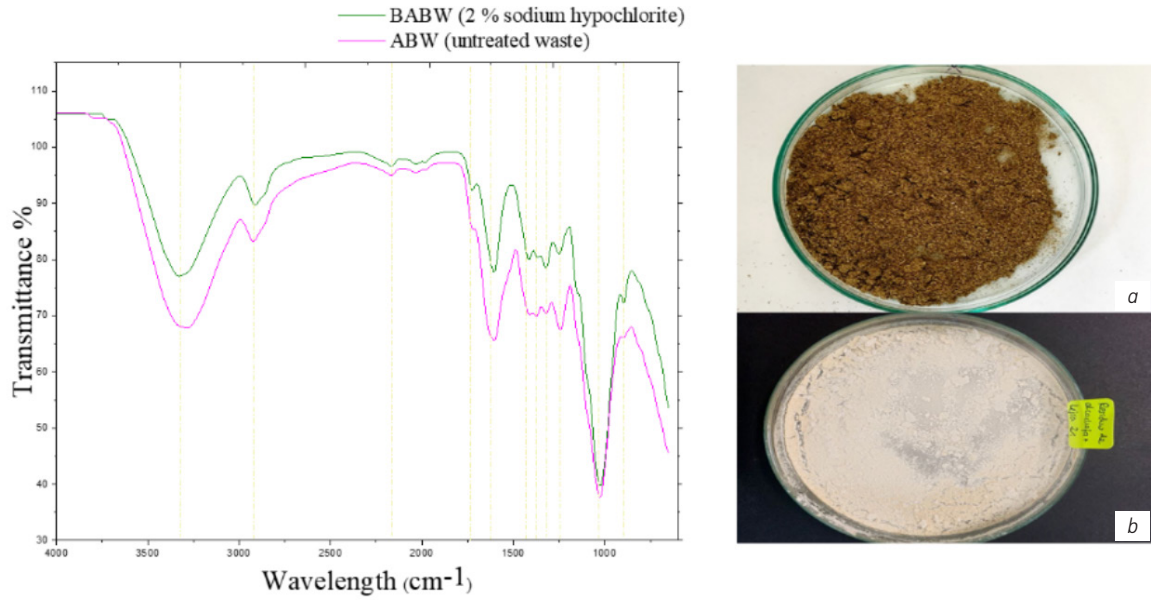


Fig. 3. *Comparative FTIR spectra for the untreated artichoke bracts waste and also treated with sodium hypochlorite at 2%: (a) artichoke bracts waste (ABW) untreated and (b) bleached with sodium hypochlorite (BABW)*



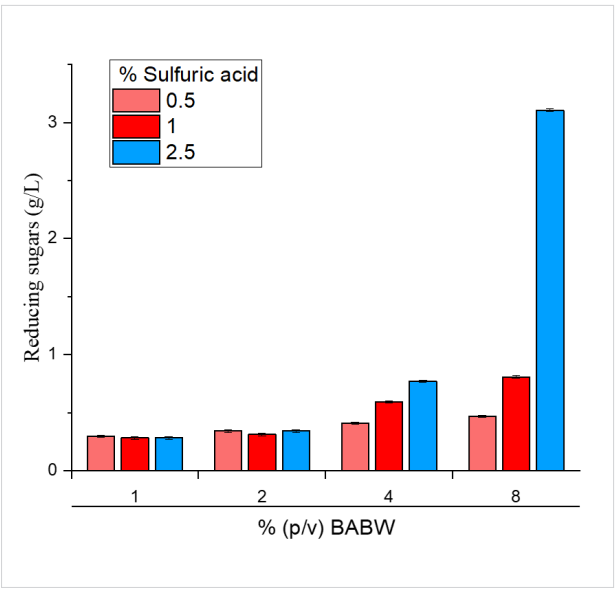
index (*Table 2*), where the ABW obtained 46.2% showing a significant difference with BABW (72.1%). The color of the vegetal waste reveals natural impurities, pigments, and lignin that stay between the cellulose fibers, this way, an elevated whiteness index would point to an increase of accessible surface to cellulose (Alghooneh et al., 2017).

Table 2. *Whiteness index (WI) for the raw artichoke waste and the waste treated with 2% sodium hypochlorite*

Waste	L*	a*	b*	WI (%)
ABW	50.1	4.9	19.6	46.2
BABW	79.1	1.8	18.2	72.1

The BABW was submitted to hydrolysis to obtain reducing sugars (RS) for fermentation (*Fig. 4*). A maximum concentration of 3.1 g/L RS was obtained applying 8% (w/w) of BABW with 2.5% of H_2SO_4; at the same time, according to Tukey test, it significantly differs from the rest of used treatments ($P < 0.05$). Similarly, Nwogwugwu et al. (2019) reported maximum values of up to 3.1 g/L of RS using waste of calabash pulp (*Crescentia cujete*). In addition, Christy et al. (2021) obtained within 0.54–36 g/L RS by applying acid hydrolysis palm waste (*Borassus flabellifer*). Moreover, it is known that a higher concentration of acid and longer treatment time may contribute to the production of furfural compounds from hydrolyzed sugars and may affect fermentation (Deshavath et al., 2017).

Fig. 4. *Impact of sulfuric acid (H_2SO_4) concentration and artichoke bleached waste (ABW) on the production of reducing sugars (g/L)*



Production of total carotenoids

The impact of pH and agitation speed was evaluated on the culture medium of hydrolyzed BABW to identify the maximum conditions in the production of carotenoids. In *Table 3*, it is observed that *Rhodotorula mucilaginosa* TQ21 at pH 5 and 120 rpm obtained high values of VCC and TFC of 2968.95 µg/L and 1228.53 µg/g, respectively, at 30°C for 72 hours. At the same time, these results exceed what was reported by Dias-Moreira et al. (2021) who obtained 331 and 255 µg/L of VCC on coffee pulp and skin waste, respectively. Similarly, Cheng and Yang (2016) achieved 2611 µg/L of VCC using molasses at pH 5.8 and 120 rpm. Notwithstanding, Machado et al. (2019) accomplished a high production of 4164.45 µg/L for 144 hours in a commercial yeast malt medium at pH 6, 130 rpm at 25°C.

The obtained data for VCC, TFC and CBC were submitted to a regression and variance analysis (ANOVA) for a first-degree model subject to pH and agitation speed with a 95% confidence level. The values for F showed to be significant to VCC, TFC, and CBC (*Table 4*). Both pH and agitation speed factors significantly influence in VCC with *P* values of 0.0011 and 0.0051, respectively.

Table 3. *Volumetric carotenoids concentration (VCC), total fraction of carotenoids (TFC) and cell biomass concentration (CBC) of the fermentation of the culture medium of hydrolyzed BABW*

Runs	Parameters		RA (%)	CBC (g/L)	FTC (µg/g)	VCC (µg/L)
	pH	rpm				
T1	5.4	91.4	67.85	2.13	746.25	1585.78
T2	6.5	120	72.94	2.54	844.10	2145.42
T3	6.5	120	72.90	2.54	458.05	1164.22
T4	7.6	91.4	82.07	3.00	357.84	1073.53
T5	7.6	148.6	70.24	3.08	589.03	1816.18
T6	6.5	120	72.74	2.54	818.71	2080.88
T7	5.4	148.6	64.48	2.90	914.47	2651.96
T8	6.5	120	73.10	2.38	912.28	2166.67
T9	5	120	84.02	2.42	1228.53	2968.95
T10	8	120	42.83	2.08	498.82	1039.22
T11	6.5	120	57.88	2.54	707.81	1799.02
T12	6.5	120	72.90	2.38	889.58	2112.75
T13	6.5	160	85.09	3.33	740.44	2468.14
T14	6.5	80	80.20	2.08	718.82	1497.55

Table 4. *Data from variance analysis (ANOVA) of VCC (µg/L), TFC (µg/g), and CBC (g/L) for Rhodotorula mucilaginosa TQ21*

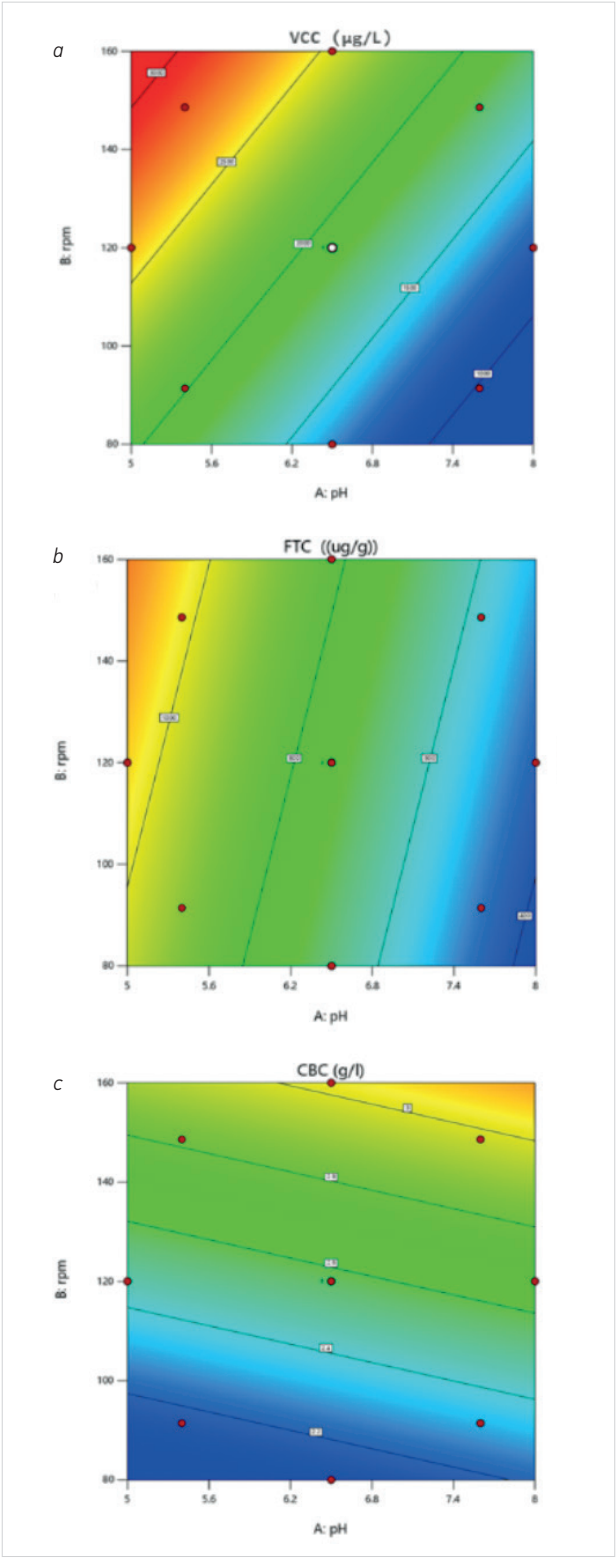
Response	VCC (µg/L)			TFC (µg/g)			CBC (g/L)		
Source	Sum of squares	F Value	*P* Value	Sum of squares	F Value	*P* Value	Sum of squares	F Value	*P* Value
Model	3.32×10^6	16.65	0.0007	4.02×10^5	11.32	0.0027	0.9059	5.38	0.026
A-pH	2.05×10^6	20.59	0.0011	3.78×10^5	21.33	0.001	0.0472	0.5607	0.4712
B-rpm	1.27×10^6	12.71	0.0051	23329.87	1.32	0.2781	0.8587	10.19	0.0096

In *Fig. 5,* it is observed that the VCC value slightly increases whenever pH decreases (until pH 5), and at the same time, the agitation speed increases. Indeed, Arias (2014) pointed out that at pH 5 higher biomass and carotenoids were obtained, which suggests that *Rhodotorula mucilaginosa* prefers slightly acid conditions. Besides, during the microbial growth, organic acids are released, and these intermediaries are assimilated again stimulating the carotenogenesis. In the same way, the agitation speed increase reflects good stimuli in VCC as much as in CBC, since it influences the nutrient distribution and the oxygen distribution in the culture medium, stimulating enzymes such as phytoene desaturase, β-carotene hydrolase, and lycopene cyclase (Borba et al., 2018). In FTC, the variation of agitation speed was not significant ($P > 0.05$); nonetheless, the pH differs positively between 5–6 values showing better pigmentation at treatment 3 with a CBC of 2.4 g/L, in spite the maximum CBC of 3.3 (Mata-Gómez et al., 2014).

Characterization of obtained carotenoids

The preliminary identification of the extracted pigment was achieved through UV-VIS spectrum by a scan within 200–800 nm, and a maximum of 500 nm was reported within regions 440–550 (*Fig. 6a*). These results are corroborated by Sharma and Ghoshal (2020b) who confirmed carotenoids presence within 400–500

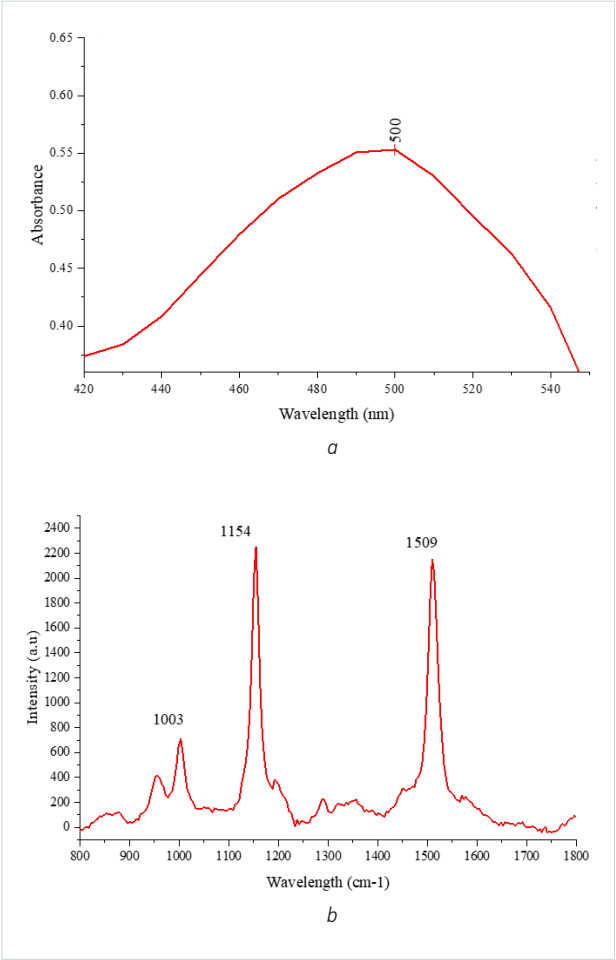
Fig. 5. *Colored contour charts showing the interaction between pH and agitation speed for maximum production of VCC (a), FTC (b), and CBC (c)*



nm, showing specific peaks of 450, 485 and 490 for beta-carotene, torulene and torularhodin, respectively. In that same way Mussagy et al. (2019) described that DMSO, the solvent used, conducts higher release rates of intracellular carotenoids, where torularhodin, the most polar carotenoid exhibits a maximum absorbance at 500 nm.

With focal RAMAN microscopy (*Fig. 6b*), the peak 1003 cm^{-1} was identified as attributable to a combination of δ (C = CH) methyl in the flat balancing and δ (C–H) flexion modalities outside the chart (Marshall and Marshall, 2010); in addition, the peak 1154 cm^{-1} assigned to v2 stretching (C–C) symmetrical and peak 1509 cm^{-1} attributable to symmetric stretching v1 (C = C) in phase (Mot et al., 2017). The results are similar to those reported by Pacia et al. (2016), who describe peaks of 1511, 1154 and 1002 cm^{-1} as features for carotenoids (beta-carotene).

Fig. 6. *Carotenoids analysis of Rhodotorula mucilaginosa TQ21 through UV-VIS spectrum (a) and RAMAN (b)*



Our preliminary results demonstrated for the first time that the *Rhodotorula mucilaginosa* TQ21 isolate presented potential in the production of total carotenoids from a culture medium based on hydrolyzed artichoke waste, in addition to the advantages that can be obtained through the design of response surface methodology to maximize VCC and FTC through optimization of pH values and stirring speed.

Conclusions

A strain of *Rhodotorula mucilaginosa* TQ21 was identified and cultivated, showing the ability to grow and produce carotenoids from artichoke hydrolyzed waste as the only carbon source. Response surface methodology was used by a central compound rotational design and optimal values of 2968.95 µg/L VCC (12258.53 µg/g of TFC) were obtained at pH 5 and 120 rpm at 30°C for 72 hours. In addition, TQ21 pigments showed typical carotenoid characteristics by their UV-VIS spectrum at 500 nm, and peaks of 1003, 1154 and 1509 cm^{-1} were observed by RAMAN analysis. The results suggest that *Rhodotorula mucilaginosa* TQ21 constitutes a promising microorganism for the commercial production of carotenoids from artichoke waste. This process means an economical way to reduce the requirement of high-cost nutrients and at the same time mitigate environmental pollution, which contributes to subsequent scaling and wide use in the global market of carotenoids for food, cosmetics and animal additives.

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