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Sustainable production of sarcinaxanthin by *Kocuria palustris* using agro-industrial residues: optimization and evaluation of photoprotective and antioxidant activities

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ABSTRACT

Carotenoids are natural pigments lipophilic natural pigments and have a wide range of biotechnological applications. This study aimed to produce carotenoids by cultivating Kocuria palustris on alternative substrates the selected condition was subsequently applied to process scale-up the selected cultivation conditions were subsequently used for process scale-up the selected cultivation conditions were subsequently used for process scale-up and to evaluate the photoprotective and antioxidant activities of the resulting extract. Production was carried out using a 2^2 factorial design to evaluate the effects of glucose and Tangerine Peel Juice (TPJ) concentrations, while maintaining a constant whey concentration, on biomass production, total carotenoids, specific carotenoids, and Sun Protection Factor (SPF). Preliminary characterization of the pigments by spectrophotometry revealed an absorption profile compatible with sarcinaxanthin. Fourier Transform Infrared Spectroscopy (FTIR) revealed functional groups consistent with carotenoid structures, while Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray Spectroscopy (EDS) provided complementary information on the morphology and elemental composition of the extract. The experimental design demonstrated that supplementation of the residual medium with glucose and *Citrus reticulata* peel significantly enhanced carotenoid production and SPF. The selected cultivation conditions were subsequently used for process scale-up, during which *K. palustris* maintained its carotenoid-producing capacity in a medium formulated with alternative agro-industrial substrates. The extract exhibited high photoprotective activity, reaching an SPF of 41.21. However, given the complex composition of the extract, this activity could not be attributed exclusively to the carotenoids produced by the bacterium. In addition, the extract exhibited antioxidant activity ranging from 31.82 to 61.36% in the β-

carotene/linoleic acid system, with no dose-dependent behavior across the concentrations evaluated. In conclusion, *K. palustris* shows potential for the sustainable production of carotenoids with a profile compatible with sarcinaxanthin using alternative agro-industrial substrates. Process scale-up and the photoprotective and antioxidant properties of the extract support the potential for further development of this bioprocess. These findings are particularly relevant to the cosmetic and biotechnology industries, where there is growing interest in natural pigments and bioactive compounds obtained through more sustainable production routes.

Keywords: Sarcinaxanthin. Photoprotection. Sustainability. Agro-industrial waste.

Introduction

Carotenoids are natural lipophilic pigments widely distributed in plants, algae, fungi, and bacteria, responsible for colors ranging from yellow to red. Structurally, they are primarily classified into carotenes—composed of carbon and hydrogen—and xanthophylls, which contain oxygenated functional groups (Rehman et al., 2020; Cheng et al., 2020). Beyond their role as pigments, these compounds exhibit biological properties that favor their application in the food, pharmaceutical, and cosmetic sectors, particularly due to their antioxidant and photoprotective activities (Meléndez-Martínez et al., 2019; Zaytseva et al., 2021; Xavier et al., 2026).

Although plants and microorganisms are important natural sources of carotenoids, microbial production has attracted growing interest due to ease of cultivation, rapid production, reduced dependence on climatic conditions, and the potential to use low-cost alternative substrates (Sengupta; Bhowal, 2023; Zhang et al., 2024; Xavier et al., 2026). Among the producing microorganisms, bacteria exhibit metabolic diversity and the ability to synthesize various bioactive carotenoids, making them promising sources for biotechnological processes (Xavier et al., 2026).

Among bacterial carotenoids, C50 carotenoids stand out, although they are considered less common than C40 carotenoids. This group includes sarcinaxanthin, a xanthophyll associated with antioxidant and photoprotective properties, showing potential for biotechnological and cosmetic applications (Netzer et al., 2010; Goksøyr, 2013; Mendes-Silva et al., 2021). The bacterium *Kocuria palustris* is notable as a producer of this pigment; its capacity to produce sarcinaxanthin and its photoprotective potential have been previously demonstrated (Mendes-Silva et al., 2021).

The photoprotective activity of these compounds is particularly relevant given the effects of excessive exposure to ultraviolet (UV) radiation, which is considered a major risk factor for skin conditions such as erythema, photoaging, and skin cancer. In this context, the use of sunscreens is a key strategy for mitigating UV-induced damage, driving interest in identifying new substances with

photoprotective properties. At the same time, there is growing interest in products of natural origin that are biodegradable and have a lower environmental impact, including for cosmetic applications and topical formulations. (Salvo et al., 2023; Sengupta; Bhowal, 2023).

This search is also linked to concerns regarding certain synthetic compounds used in these formulations. Some UV filters—such as oxybenzone, octinoxate, and octocrylene—have been detected in marine environments and are being investigated for their potential effects on aquatic organisms and reef ecosystems (MILLER et al., 2021; Reis-Mansur et al., 2023; Hodge et al., 2025). In this context, microbially derived carotenoids emerge as bioactive compounds of interest for the development of products with photoprotective and antioxidant potential (Xavier et al., 2026).

However, beyond the product's biological properties, the sustainable production of these pigments is a key factor for their biotechnological exploitation. Costs associated with culture media can limit large-scale microbial production; thus, utilizing agro-industrial waste offers an alternative for supplying nutrients, adding value to by-products, and reducing environmental impact (Mata-Gómez et al., 2014; Ram et al., 2020; Xavier et al., 2026). Substrates of interest include *Citrus reticulata* peel—which contains carbohydrates and other organic constituents—and whey, a source of lactose, proteins, and minerals (Gondim et al., 2005; Andrade et al., 2016; Javed et al., 2019). Supplementation with glucose, a readily assimilable carbon source, can also contribute to the medium's nutritional composition, with its concentration serving as a factor to consider during process optimization.

Despite advances in using agro-industrial waste for microbial pigment production, information regarding the combined use of *C. reticulata* peel, whey, and glucose for carotenoid production by *K. palustris* remains limited. Although culture condition optimization and bioreactor scale-up have previously been employed to enhance carotenoid production by this species (Mendes-Silva et al., 2021), the use of alternative substrates expands the potential for developing more sustainable processes.

Given this context, the present study aimed to evaluate and optimize carotenoid production by *K. palustris* using *C. reticulata* peel, whey, and glucose as culture medium components, to scale up the process, and to evaluate the photoprotective and antioxidant activities of the resulting extract.

Materials and methods

Bacterial strain

The bacterium *Kocuria palustris* FT-7.22, used in this study, was previously isolated and identified by Mendes-Silva et al. (2021) from soil in the Caatinga biome, collected at the Fazenda Tamanduá Private Natural Heritage Reserve (RPPN) in the municipality of Santa Terezinha, Paraíba (7°2′20″ S and 37°26′43″ W). Identification was initially performed via morphological characterization using Gram staining and confirmed at the molecular level through 16S rRNA gene

sequencing, showing 99% similarity to *K. palustris* sequences. The sequence of isolate FT-7.22 is deposited in GenBank under accession number KX990292 (Mendes-Silva et al., 2021).

Cultivation conditions

Pure cultures of *K. palustris* were transferred to 250 mL Erlenmeyer flasks containing 50 mL of TSB medium (17.0 g casein, 3.0 g soy, 5.0 g NaCl, 2.5 g K_2HPO_4, 2.5 g dextrose per 1000 mL distilled water). Pre-inoculum cells were cultivated under agitation at 30°C (250 rpm) in a shaking incubator (TECNAL TE-420, São Paulo, Brazil) for 24 h. Initial growth kinetics were determined using a standardized Optical Density (OD) measurement of the cell suspension (OD at 600 nm) of 0.1.

Bacterial pigment production using agro-industrial residue

Microbial fermentation was carried out in culture media with varying compositions—specifically *C. reticulata* fruit peel, glucose, and whey—with the whey content remaining constant. Culture conditions were maintained at optimized levels (250 rpm, 30°C, pH 7.0), following Mendes-Silva (2021).

C. reticulata fruit peels, obtained from a commercial center in the city of Recife, were washed under running water and separated from the pulp. Subsequently, 100 g of fresh peels were homogenized with 500 mL of distilled water in a food processor to prepare the broth. The broth was then filtered—first through a sieve and then through gauze—and sterilized according to the methodology described by Berger et al. (2018).

The whey (bovine origin) was obtained from the Cachoeirinha farm in the municipality of Garanhuns, Pernambuco (08°49'38.81" S; 36°34'24.65" W) and sterilized for use. Finally, the glucose was obtained from a commercial manufacturer (Sigma-Aldrich).

A full 2^2 factorial design was conducted to investigate the effects of glucose and Tangerine Peel Juice (TPJ) substrate concentrations on biomass production, total carotenoids, specific carotenoids, and SPF after 48 h—considered as response or dependent variables. To this end, seven experimental runs were performed, evaluating each independent variable at three levels: minimum (-1), central (0), and maximum (+1), as shown in Table 1. The obtained data were analyzed using Statistica® software, version 12.0 (StatSoft Inc., USA), testing the significance of the results ($p < 0.05$).

Table 1

Levels of independent variables used in factorial design experiments.

Variables	Levels		
	-1	0	+1

Glucose (g)	0	0,25	0,5
Tangerine Peel Juice (mL)	0	11,5	23

Determination of biomass

To determine the biomass dry weight (g/L), the culture was centrifuged at 12,000 rpm and 5°C for 7 min. The resulting supernatant was transferred to a Falcon tube for subsequent analysis, while the cell pellet was frozen for 12 h and then dried in a freeze-dryer (Terroni Enterprise II, São Paulo, Brazil) at 60°C for 48 h. The resulting biomass was quantified in g/L.

Extraction and quantification of carotenoids

Carotenoids were extracted from the dried biomass according to the method described by Arulselvi et al. (2013), with the following modifications: vortexing (Phoenix/AP 56, São Paulo, Brazil) for 30 s, a 2-minute ultrasonic bath (Unique/USC 800, São Paulo, Brazil), and centrifugation at 14,000 rpm for 7 min (HERMLE Z383K, Wehingen, Germany). Subsequently, the supernatant was transferred to another tube, and the process was repeated until no further pigment was extracted from the dried biomass.

The total carotenoid concentration was determined spectrophotometrically and calculated according to the formula proposed by Davies (1976). To this end, the absorbance of the broth was measured at the wavelength of maximum absorption (439 nm) using a Bioespectro SP-220 spectrophotometer (Bioespectro, Paraná, Brazil). The concentration was calculated using the absorbance (A), the volume of solvent used for extraction (y), and the absorption coefficient $A_{1cm}^{1\%}$ = 2500 (Britton, 1985), in accordance with Equations 1, 2, and 3. Total carotenoid content was expressed in µg/L, and specific carotenoids in µg/g.

$$\mu g = \frac{A \,.y\,(mL).\ 10^6}{A_{1\,cm}^{1\%}.100} \qquad \text{Equation 1}$$

Where: A = maximum absorbance, y= the volume of solvent used in the extraction, $A1cm$ 1% = the absorption coefficient equivalent to 2500.

$$Specific\ carotenoids\ (\mu g/g) = \frac{\mu g}{sample\ weight\ (g)} \qquad \text{Equation 2}$$

$$Total\ carotenoids\ \mu g/L = Biomass\ dry\ weight(g/L)\ .spcecific\ carotenoids\ (\mu g/g) \qquad \text{Equation 3}$$

Production kinetics

Production kinetics were evaluated over 120 h of cultivation, with analyses performed at 0, 24, 48, 72, 96, and 120 h. Based on the monitoring of cell growth throughout this period, the maximum microbial growth rate (µmax) was determined. Carotenoid and pigment production were also estimated using the methodology described by Aksu and Eren (2007).

Process scale-up

After identifying the optimal experimental conditions, production was scaled up to 1.6 L using eight 1 L Erlenmeyer flasks, each containing 200 mL of culture medium, while maintaining the 20% ratio used in the initial assays. Fermentation was carried out for 48 h at 30 °C and 250 rpm, with an initial pH of 7.0.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra were obtained using a Bruker Vertex 70 FTIR spectrometer equipped with an Attenuated Total Reflectance (ATR) accessory with a diamond crystal.

The spectra were recorded in the 400–4000 cm^{-1} spectral range, with a resolution of 4 cm^{-1} and 16 scans per sample. The samples were analyzed directly on the ATR crystal, without prior treatment. Subsequently, the obtained data were processed using OPUS software, including baseline correction and normalization when necessary..

Scanning Electron Microscopy (SEM) with Energy-Dispersive X-ray Spectroscopy (EDS)

For SEM and EDS analyses, the lyophilized extract was prepared through the following steps: deposition of the sample onto an aluminum stub using conductive carbon tape, removal of excess material to obtain a homogeneous layer, and subsequent gold coating to impart electrical conductivity to the sample surface during analysis.

Subsequently, images were acquired using secondary electrons at various magnifications and a resolution of 1024 × 1024 pixels, employing an Everhart-Thornley detector coupled to a TESCAN Mira 4 LMU scanning electron microscope. Acquisitions were performed at an accelerating voltage of 10 kV.

For EDS chemical mapping, the beam accelerating voltage was increased to 20 kV to enable the excitation and detection of all elements of interest, should they be present. Under these conditions, elemental maps with a resolution of 768 × 768 were collected from the regions of interest.

In vitro photoprotective activity

SPF determination is a technique used to verify the efficacy of sunscreens regarding UVB protection within the electromagnetic spectrum, as this range is responsible for causing skin erythema (Araujo & Souza, 2008). Accordingly, methanolic solutions at a concentration of 100 mg/L were prepared in triplicate using the extracted pigment, which had been produced on a large scale. Subsequently, absorbance was measured using a spectrophotometer at wavelengths ranging from 290 to 320 nm. The results were used to calculate the SPF according to the described methodology. described by Velasco et al. (2011), using [Equation 4] shown below.

$$FPS = FC.\sum_{290}^{320}.EE\ (\lambda).I\ (\lambda).Abs\ (\lambda)$$ Equation 4

Where: FC = correction factor (=10); EE (λ) = erythemal effect radiation wavelength (λ); I (λ) = sunlight intensity at a wavelength (λ); and Abs (λ) = sample absorbance at wavelength (λ).

In vitro antioxidant activity

The $^1O_2$ scavenging capacity was measured by monitoring the effect of the carotenoid extract on the oxidation (bleaching) of β-carotene induced by the oxidative degradation products of linoleic acid, following the methods of Rufino et al. (2006) and Duarte Almeida et al. (2006). The extracts were diluted to concentrations ranging from 0.61 to 4.93 mg/mL. Approximately 25 µL of the samples were added to 1 mL of the β-carotene/linoleic acid emulsion, and subsequently, 175 µL of this mixture was placed in a microplate. Measurements were taken at time zero (t0) at 470 nm; the samples were then incubated for 120 min at 50 °C, and the measurement was recorded again at that time (t120). All analyses were performed in triplicate using BHT (butylated hydroxytoluene) (Sigma, São Paulo, Brazil) as a standard. The inhibition was calculated as described in [Equation 5].

$$\%\ inibição = \left[\frac{(abs\ sistema - abs\ amostra)}{abs\ sistema}\right] x\ 100$$ Equation 5

Results and discussion

Effect of glucose and TPJ on the cultivation of *K. palustris* for carotenoid production

To evaluate the influence of culture medium supplementation, a 2^2 factorial design was employed, using glucose concentration and TPJ extract volume as independent variables. The factors were coded at three levels: -1 (low level), 0 (central level), and +1 (high level). For glucose, the levels corresponded to the addition of 0 g, 0.25 g, and 0.50 g, respectively. For TPJ, the levels corresponded to the addition of 0 mL, 11.5 mL, and 23 mL, respectively, while the whey volume was kept constant at 27 mL in all assays. The results obtained for each experimental condition are presented in Table 2.

Table 2 A 2^2 factorial design applied to evaluate the effects of glucose and TPJ concentrations on the production of biomass, carotenoids, and SPF by *K. palustris*.

ENSAIO	GLICOSE (%)	SCT (%)	BIOMASSA (g/L)	CAROTENOIDES TOTAIS (µg/L)	CAROTENOIDES ESPECÍFICOS (µg/g)	FPS
1	-1	-1	3,14	884,00	281,53	2,24
2	+1	-1	3,53	3.411,99	966,57	8,97
3	-1	+1	3,88	4.591,99	1185,03	20,76
4	+1	+1	3,62	6.688,00	1850,07	30,39
5	0	0	3,85	4.328,00	1330,91	18,21
6	0	0	3,41	4.827,98	1417,91	18,03
7	0	0	3,65	4.760,00	1304,11	16,78

ASSAY	GLUCOSE	TPJ	BIOMASS	TOTAL CAROTENOIDS	SPECIFIC CAROTENOIDS	SPF
1	-1	-1	3,14			
2	+1	-1	3,53			
3	-1	+1	3,88			
4	+1	+1	3,62			
5	0	0	3,85			
6	0	0	3,41			
7	0	0	3,65			

Note: the coded levels (-1, 0, and +1) corresponded to glucose concentrations of 0 g, 0.125 g, and 0.250 g, respectively, and to TPJ extract volumes of 0 mL, 11.5 mL, and 23 mL, respectively. In all assays, the whey volume was kept constant at 27 mL.

The results demonstrated that biomass production showed no direct relationship with carotenoid production. Trial 3 yielded the highest biomass concentration (3.88 g/L), whereas the highest values for total (6,688 µg/L) and specific (1,850.07 µg/g) carotenoids were observed in Trial 4, which had a biomass of 3.62 g/L. A similar pattern was observed by Mendes-Silva et al. (2021) in *K. palustris*; they found an inverse relationship between cell growth and specific carotenoid production. These results support the findings of Buzzini et al. (2005), who reported that maximum biomass and carotenoid values do not necessarily show a direct correlation.

In this context, although biomass production is linked to growth and cell multiplication (Madigan et al., 2021), its magnitude can vary depending on the nutritional composition of the medium, the availability of carbon and nitrogen sources, and the process operating conditions (Stanbury; Whitaker; Hall, 2017). Recent studies also demonstrate that the nature and availability of nutrients influence microbial biomass growth and yield (Sakarika et al., 2023; Golan et al., 2025).

Regarding carotenoid production, Mendes-Silva et al. (2021) highlight specific production as a relevant parameter for assessing the synthesis of these pigments in relation to the biomass produced. In this regard, the results obtained reinforce the importance of this parameter, since the experimental run with the highest specific carotenoid contente velasco did not correspond to the one yielding the

highest biomass. Furthermore, the condition containing the highest levels of TPJ and glucose simultaneously exhibited the highest values for total carotenoids, specific carotenoids, and volumetric productivity. Conversely, assay 1—in which glucose and TPJ were absent and only whey remained as the medium's substrate—showed the lowest specific carotenoid production (281.53 μg/g). Thus, the combination of TPJ and glucose at their maximum evaluated levels (23 mL and 0.25 g, respectively) proved promising for carotenoid production by *K. palustris*.

The influence of glucose concentration on carotenoid production has also been observed in other carotenogenic microorganisms. Given the 50 mL culture volume used in this study, the amounts of 0, 0.125, and 0.250 g corresponded to glucose concentrations of 0, 2.5, and 5.0 g/L, respectively. In *Kocuria marina* DAGII, Mitra et al. (2016) observed an increase in β-cryptoxanthin production as glucose concentration rose to 7.5 g/L—reaching a yield of 3.6 mg/L—with no significant further increase at higher concentrations. Conversely, for *Kocuria* sp. RAM1, glucose (evaluated within the 0.5–1.5 g/L range) was not among the significant factors selected for the subsequent carotenoid production optimization stage (Metwally et al., 2022). These results indicate that the response to glucose supplementation can vary depending on the species, the concentration used, and other cultivation conditions. In the present study, within the evaluated range, the highest glucose concentration (5.0 g/L), combined with the highest TPJ level, resulted in the highest total and specific carotenoid production.

The available literature on carotenoid production by *K. palustris* using agro-industrial residues remains limited. Mendes-Silva et al. (2021) reported high sarcinaxanthin production using TSB medium, whereas the use of residues as a nutrient source remains underexplored. In this context, the present study expands the cultivation options for the species by demonstrating carotenoid production in a medium containing whey and tangerine peel juice.

The composition of orange peel (*Citrus* sp.) has been documented, revealing the presence of soluble sugars—such as glucose, sucrose, fructose, and xylose—as well as insoluble polysaccharides like pectin, cellulose, and hemicelluloses (Rivas et al., 2008; Javed et al., 2019; Kuo et al., 2019). Rivas et al. (2008) evaluated its use in fermentation by *Aspergillus niger* for citric acid production. Similarly, Kuo et al. (2019) investigated its utilization for bacterial cellulose production by *Gluconacetobacter xylinus*, while Javed et al. (2019) highlighted the use of citrus waste as a raw material in enzymatic and fermentation processes. In experiments conducted by Gondim et al. (2005), the authors assessed the proximate composition of various fruit peels (avocado, pineapple, banana, papaya, passion fruit, melon, and tangerine); their results showed that tangerine peel stood out among those evaluated due to its high carbohydrate content.

In addition to citrus waste, residues from other fruits have been investigated as alternative carbon sources for bacterial cultivation. A recent study utilized hydrolysates from banana, mango, orange, and pineapple peels to cultivate

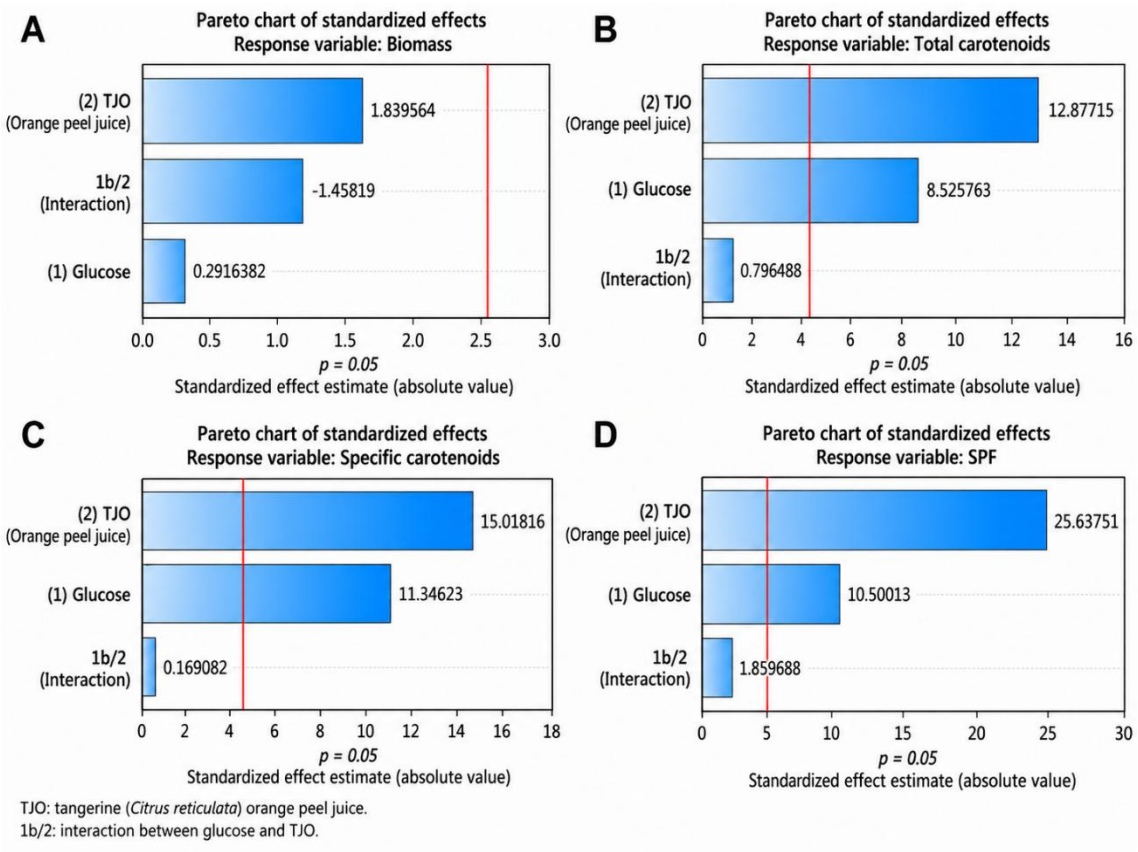
Halomonas alkalicola, demonstrating the potential of these residues as substrates for biotechnological processes (Muigano et al., 2024). Specifically regarding carotenoids, Elsanhoty et al. (2012) used date (Phoenix dactylifera) waste syrup to cultivate *Lactobacillus plantarum* QS3, obtaining 16.21 mg/kg of dry cells—a value that reached 54.89 mg/kg following supplementation and optimization of cultivation conditions. These studies demonstrate the potential of various fruit wastes as carbon sources for bacterial cultures and, particularly, for carotenoid biosynthesis.

In this context, the use of TPJ in the present study aligns with strategies to valorize fruit waste as alternative substrates for bacterial carotenoid production. Whey, included as a component of the culture medium, served as an additional nutrient source for *K. palustris*, owing—among other factors—to the presence of lactose in its composition (ANDRADE et al., 2016).

The Pareto charts shown in Figure 1 illustrate the influence of glucose and TPJ concentrations on the production of biomass, total carotenoids, specific carotenoids, and SPF. None of the variables showed a significant effect on biomass production, as can be seen in Figure 1A. However, both glucose and TPJ demonstrated a statistically significant positive influence on the production of total carotenoids (Figure 1B), specific carotenoids (Figure 1C), and SPF (Figure 1D); this indicates that increasing the concentration of each substrate individually enhances pigment production and stimulates an increase in SPF.

In the present study, results from the experimental design showed variations in biomass and carotenoid production across the evaluated conditions. Biomass ranged from 3.14 to 3.88 g/L, with the highest value observed in assay 3 (3.88 g/L). Total carotenoid values ranged from 884.00 to 6,688.00 µg/L, with the highest production in assay 4 (6,688.00 µg/L), which yielded a biomass of 3.62 g/L. This same assay also yielded the highest values for specific carotenoids (1,850.07 µg/g) and SPF (30.39).

Fig. 1 Pareto charts obtained from the results of the 2^2 factorial design applied to investigate the production of biomass (A), total carotenoids (B), specific carotenoids (C), and SPF (D) by *K. palustris*.



In the present study, glucose and TPJ served as the independent variables in the factorial design, while biomass, total carotenoids, specific carotenoids, and SPF were considered response variables. Analysis of the Pareto charts demonstrated that glucose and TPJ exerted distinct effects on these responses. Regarding biomass production (Figure 1A), neither factor exceeded the threshold for statistical significance ($p = 0.05$); standardized effects of 1.84 for TPJ and 0.29 for glucose were observed. The interaction between the factors was also not significant (1.46), indicating that, under the conditions evaluated, variations in glucose and TPJ did not lead to statistically significant changes in biomass production.

In contrast, for total carotenoids (Figure 1B), TPJ and glucose showed significant effects, with standardized magnitudes of 12.88 and 8.53, respectively; the standardized effect of TPJ was approximately 1.5 times greater than that of glucose. The interaction between the factors showed a lower magnitude (0.80) and was not statistically significant. A similar pattern was observed for specific carotenoids (Figure 1C), where TPJ showed a standardized effect of 15.02 and glucose 11.35, while the interaction (0.17) was not significant.

For SPF (Figure 1D), TPJ showed the standardized effect of greatest magnitude (25.64), followed by glucose (10.50)—both statistically significant—whereas the interaction between the factors was not significant (1.86). These results demonstrate that TPJ was the factor with the greatest influence on total and specific carotenoid production and on SPF, while biomass did not respond

significantly to variations in the factors studied. Beyond statistical significance, the results hold practical relevance, as the condition containing the highest evaluated levels of TPJ and glucose yielded the highest values for total carotenoids (6,688.00 µg/L), specific carotenoids (1,850.07 µg/g), and SPF (30.39).

The absence of a significant effect on biomass, alongside the significant effects observed for carotenoid production, demonstrates that cell growth and the synthesis of these pigments did not respond in the same way to the conditions evaluated. Regarding the literature, Mendes-Silva et al. (2021), when evaluating the effects of temperature and light intensity on carotenoid production by *K. palustris* in TSB medium, reported mean values of 1,692 µg/L for total carotenoids and 3.58 g/L for dry biomass. In the present study, the highest total carotenoid production was 6,688.00 µg/L, with a biomass of 3.62 g/L, demonstrating that *K. palustris* was also capable of producing carotenoids in a medium containing alternative agro-industrial substrates. Although the values obtained were higher than those reported by Mendes-Silva et al. (2021), his comparison should be considered in light of the differences in medium composition and experimental conditions employed in the two studies.

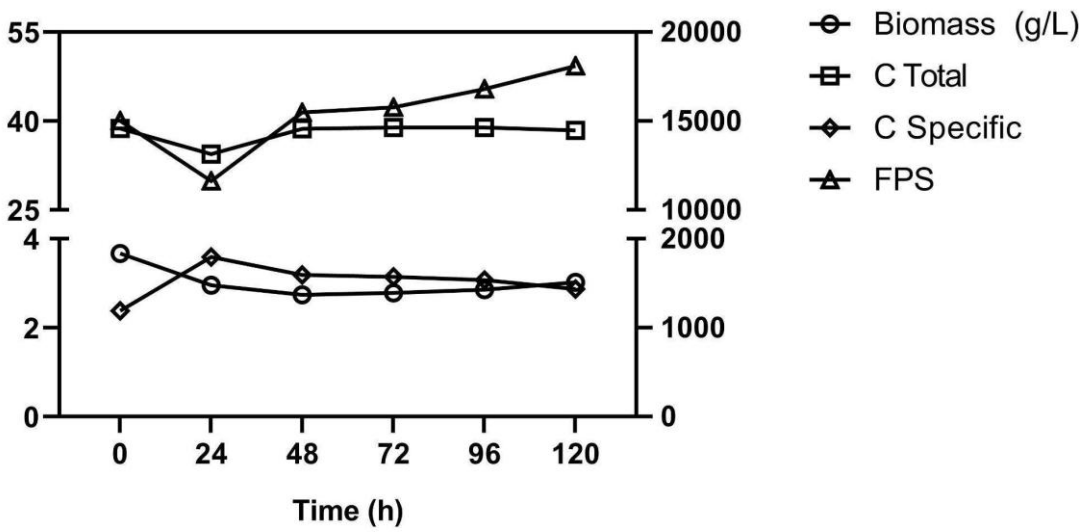
The influence of cultivation variables on carotenoid production has also been investigated in other bacterial systems using experimental design approaches. Hegazy et al. (2024) evaluated whey concentration, inoculum size, pH, temperature, and agitation speed as independent variables, using carotenoid production by *Micrococcus luteus* as the response variable. Meanwhile, Noby et al. (2023) assessed $MgSO_4$ and whey concentrations as well as inoculum size, with the production of bacterioruberin and its derivatives by *Arthrobacter agilis* NP20 serving as the response. Hussain et al. (2024) evaluated the proportion of orange peel hydrolysate, nitrogen source concentration, and temperature as independent variables to optimize lycopene production by recombinant *Escherichia coli*. These studies demonstrate that both the nutritional composition of the medium and the cultivation conditions are significant variables in the assessment and optimization of bacterial carotenoid production.

In addition to carotenoids, fruit residues have also been used as substrates in other bacterial bioprocesses. Rovina et al. (2018) used orange peel waste as a substrate for biosurfactant production by *Bacillus subtilis*, while Sarkar et al. (2020) evaluated the use of banana peel as a substrate for ethanol production by *Klebsiella* sp. These studies expand the evidence regarding the potential of fruit residues as alternative substrates in bacterial biotechnological processes.

Production kinetics

The results regarding growth kinetics, carotenoid production, and EPS after 120 hours of cultivation are shown in Figure 2.

Fig. 2 Biomass, carotenoid production, and EPS during process scale-up by *K. palustris* under optimized conditions (250 rpm, 30°C, initial pH 7.0) in residual medium.



The kinetics of the parameters evaluated over the 120-hour cultivation period are presented in Table S1 of the Supplementary Material. Biomass showed a reduction during the first 48 hours, followed by a gradual increase until the end of the cultivation. Total carotenoids exhibited an initial drop, recovering after 48 hours and remaining practically constant until 120 hours. In contrast, specific carotenoids peaked within the first 24 hours and decreased progressively during subsequent periods. SPF displayed a behavior distinct from the other parameters, decreasing during the first 24 hours of cultivation and gradually increasing from 48 hours onwards, reaching its highest value at the end of the 120-hour cultivation period.

The high dry mass value observed at the initial time point (3.672 g/L) may be attributed to biomass from the inoculum and the recovery—during centrifugation—of insoluble components present in the residual medium containing TPJ; thus, it does not represent cellular biomass alone. The reduction observed within the first 48 hours may be associated with the adaptation period of *K. palustris* to the medium's nutritional conditions, during which metabolic adjustments necessary for utilizing available substrates take place (Rolfe et al., 2012; Madigan et al., 2021). Subsequently, a gradual recovery of biomass was observed, rising from 2.744 g/L at 48 hours to 3.023 g/L at 120 hours—an increase of 10.17%—suggesting a resumption of culture growth following the initial adaptation phase.

During the kinetic study, it was observed that the increase in biomass was accompanied by a rise in SPF throughout the cultivation period. Although a gradual recovery was subsequently observed, the increase in biomass occurred concomitantly with a reduction in specific carotenoid production, highlighting distinct behaviors between these parameters. Total carotenoid production, in turn, remained practically constant during this period, not keeping pace with the increase in biomass (Figure 2).

The concentration of carotenoids present is influenced by various factors, such as extraction conditions, orange species, harvest time, cultivation location, and

storage conditions (Anticona et al., 2021). Furthermore, in general, there is no direct correlation between maximum total carotenoid values and maximum biomass production (Buzzini et al., 2005). In a study of the yeast *Phaffia rhodozyma* NCHU-FS501 in batch culture—using a medium formulated with glucose as the carbon source and peptone as the nitrogen source—Fang and Chiou (1996) observed that maximum carotenoid production occurred at a different time (35 h) than maximum cell growth (25 h).

Mendes-Silva et al. (2021) observed that *K. palustris* did not fully consume the nitrogen source during carotenoid production in a synthetic medium, indicating that this nutrient was not limiting to the process. Similarly, Orosa et al. (2005), studying *Haematococcus pluvialis*, found that nitrogen availability had little influence on carotenoid production. Given this evidence from the literature and the focus of the present study on evaluating alternative carbon sources, nitrogen consumption was not monitored.

From an industrial application perspective, the results are relevant for determining the cultivation time. Although specific carotenoid production peaked at 24 hours, total production recovered starting at 48 hours and remained virtually constant thereafter, indicating that extending cultivation to 120 hours did not result in a proportional increase in carotenoid production. This behavior suggests the possibility of reducing process time, potentially lowering operating costs. However, optimization studies and techno-economic assessments are required to establish the most suitable cultivation time for industrial-scale production.

Large-scale pigment production

Based on previously established optimal parameters, the culture was scaled up over a 48-hour period. At the end of this period, 2.9809 g of dry biomass was obtained, from which the pigments were extracted. The results demonstrated that *K. palustris* maintained its growth and carotenoid production capabilities under the conditions used for scale-up, indicating the potential for using a medium formulated with alternative agro-industrial substrates in larger culture volumes. However, assessing industrial applicability requires further studies on productivity, pigment recovery and purification, as well as a techno-economic analysis of the process.

This behavior aligns with literature reports demonstrating the influence of culture medium composition on carotenoid production. The availability of carbon and nitrogen sources, mineral salts, and other nutrients can alter both the type and concentration of the synthesized pigments. Mendes-Silva et al. (2021), for instance, produced carotenoids using *K. palustris* in a synthetic medium. Similarly, Valduga et al. (2009) used a molasses-based medium for carotenoid production by yeasts, while Mata-Gómez et al. (2014) highlight the use of agro-industrial residues as alternative carbon sources for obtaining these pigments.

Scaling up the process using a medium formulated with agro-industrial substrates enabled the continued cultivation of *K. palustris* and the acquisition of sufficient material for the subsequent stages of carotenoid extraction and

characterization. These results demonstrate the experimental feasibility of using the alternative medium at larger cultivation volumes. However, determining carotenoid productivity and recovery yields requires the standardization of extraction and purification steps—an aspect particularly relevant for the future assessment of the process's industrial viability.

Mendes-Silva et al. (2021) previously demonstrated the feasibility of scaling up carotenoid production by *K. palustris* using a synthetic medium in a 10 L bioreactor. In the present study, sustaining carotenoid production in a medium formulated with alternative agro-industrial substrates expands this perspective, indicating the potential for using these substrates at larger cultivation scales. Nevertheless, quantitative comparisons between processes must take into account differences in medium composition, cultivation systems, and pigment recovery procedures.

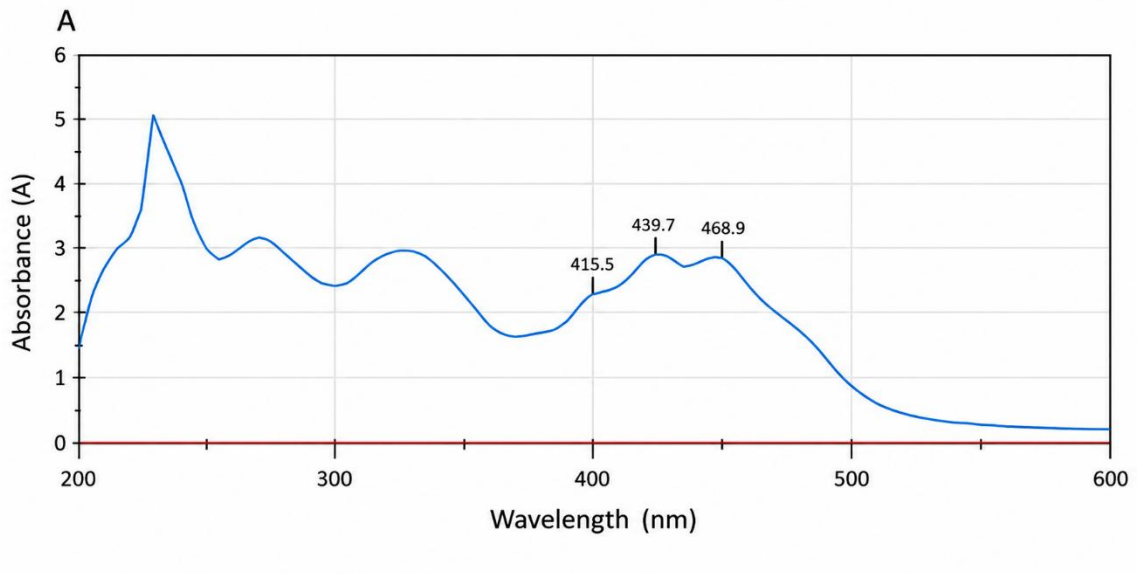
The influence of cultivation conditions on carotenoid production during scale-up has also been demonstrated in other bacterial systems. Rostami et al. (2014), using *Dietzia natronolimnaea* HS-1 in a batch bioreactor, evaluated D-glucose concentration, inoculum size, and aeration rate as independent variables to optimize carotenoid production. The estimated optimal conditions were 23.38 g/L D-glucose, an inoculum of 31.2 × 10^9 CFU/mL, and an aeration rate of 7.85 L/L·min, resulting in predicted yields of 5.48 mg/L total carotenoids and 4.99 mg/L canthaxanthin.

Taken together, these studies demonstrate that optimizing cultivation conditions and scaling up in bioreactors are efficient strategies for increasing carotenoid productivity across different microorganisms. In the present study, in addition to the high yield achieved, large-scale production provided a sufficient quantity of pigment for the extraction, purification, and subsequent identification of the produced carotenoids.

Spectral characterization of carotenoids

Spectral analysis of the pigment extract, performed in the 200–600 nm range, revealed three absorption maxima at 415.5, 439.7, and 468.9 nm (Figure 3). The observed spectral profile is consistent with that described for sarcinaxanthin—a C50 carotenoid produced by pigmented bacteria—including its glycosylated derivatives (NETZER et al., 2010). However, the similarity of the absorption profile does not allow for the structural confirmation of these compounds; the use of complementary analytical techniques, such as mass spectrometry and/or nuclear magnetic resonance, is required for their unequivocal identification.

Fig. 3 Absorption spectra of the main carotenoids of *K. palustris* identified using spectral data.



Identifying these carotenoids is significant because it allows the extract's chemical profile to be linked to the functional properties evaluated in this study. Sarcinaxanthin is a C50 carotenoid associated with antioxidant activity and protection against oxidative and photo-induced stress—characteristics that underscore its biotechnological interest. Its glycosylated derivatives, such as sarcinaxanthin monoglucoside and diglucoside, are also significant; they are part of the carotenoid profile produced by these bacteria and possess structural modifications that can influence the pigments' physicochemical properties. Thus, characterizing sarcinaxanthin and its derivatives helps elucidate the extract's composition and its antioxidant and photoprotective properties, reinforcing its potential for biotechnological application.

Beyond its functional relevance, the biosynthesis of sarcinaxanthin in *Micrococcus luteus* was described by Netzer et al. (2010). The authors characterized the intermediate carotenoids involved in this biosynthetic pathway, in which lycopene serves as the precursor for the formation of sarcinaxanthin and its glycosylated derivatives. This biosynthetic profile provides further support for interpreting the carotenoids detected in the present study.

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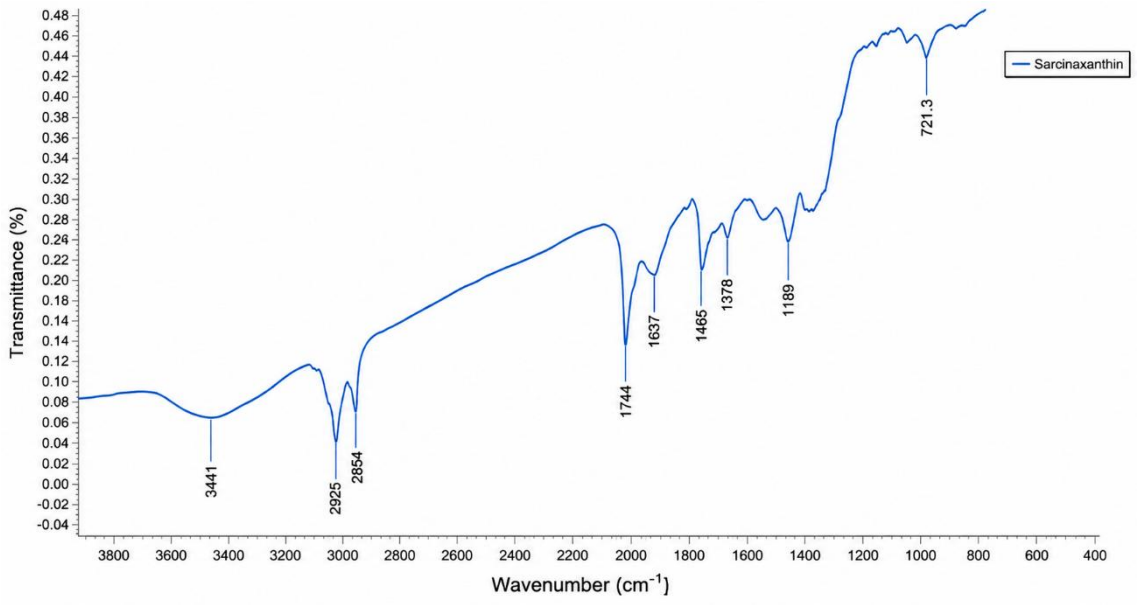
Sarcinaxanthin has been identified in various bacterial species. Schmidt et al. (2021), for instance, detected this carotenoid in *M. luteus* isolates from Caatinga soil. The strains, cultured in synthetic medium, produced sarcinaxanthin and its derivatives, exhibiting retention peaks similar to those observed in the present study. Similarly, Mendes-Silva et al. (2021) identified sarcinaxanthin in *Kocuria palustris*, a species also used in the present study. The authors observed the same three chromatographic peaks found in this research. Furthermore, the *K. palustris* isolate FT-7.22 used by those authors showed a sarcinaxanthin concentration similar to that obtained in the present work (2500 µg/g). This similarity was also observed in other carotenoid-producing bacteria, such as the genetically modified *E. coli* strain studied by Netzer et al. (2010), which produced sarcinaxanthin at levels close to those found here.

A sarcinaxantina tem sido identificada em diferentes espécies bacterianas. Schmidt et al. (2021), por exemplo, detectaram esse carotenoide em isolados de *M. luteus* provenientes do solo da Caatinga. As cepas, cultivadas em meio sintético, produziram sarcinaxantina e seus derivados, apresentando picos de retenção semelhantes aos observados no presente estudo. De forma semelhante, Mendes-Silva et al. (2021) identificaram a sarcinaxantina em *Kocuria palustris*, espécie utilizada no presente estudo. Os autores observaram os mesmos três picos cromatográficos encontrados nesta pesquisa. Além disso, o isolado *K. palustris* FT-7.22 empregado por esses autores apresentou concentração de sarcinaxantina semelhante à obtida no presente trabalho (2500 µg/g). Essa semelhança também foi observada em outras bactérias produtoras de carotenoides, como a cepa de *E. coli* geneticamente modificada estudada por Netzer et al. (2010), que apresentou produção de sarcinaxantina em níveis próximos aos aqui encontrados.

Characterization of the carotenoid extract by FTIR

The characterization of the *K. palustris* carotenoid extract by FTIR was performed to identify the main functional groups present in the sample. The obtained spectrum is shown in Figure 4.

Fig. 4 FTIR spectrum of the carotenoid extract produced by *Kocuria palustris*, highlighting functional groups characteristic of the carotenoid structure.



The FTIR spectrum of the extract displayed bands at 3441, 2925, 2854, 1744, 1637, 1465, 1378, 1189, and 921 cm^{-1}. The broad band at 3441 cm^{-1} is consistent with O–H stretching vibrations, while the bands at 2925 and 2854 cm^{-1} are associated with C–H stretching in aliphatic chains. The band observed at 1637 cm^{-1} can be attributed to C=C vibrations of unsaturated structures, whereas the bands at 1465 and 1378 cm^{-1} are consistent with methylene and methyl group deformations. The signal at 1189 cm^{-1} may be related to vibrations involving C–O bonds, and the band at 921 cm^{-1} to out-of-plane deformations of =C–H bonds. Additionally, the band at 1744 cm^{-1} is consistent with C=O stretching and may be related to the presence of other components co-extracted with the carotenoids.

Taken together, the observed bands indicate the presence of functional groups consistent with carotenoid structures, particularly regarding vibrations associated with C=C bonds and oxygenated groups. The system of conjugated unsaturations is a structural characteristic of carotenoids, while the presence of oxygenated groups aligns with xanthophyll-class carotenoids. When analyzed alongside the UV-Vis absorption profile and data reported for carotenoids produced by *Kocuria*, the FTIR profile provides evidence consistent with the presence of sarcinaxanthin in the extract (Netzer et al., 2010; Mendes-Silva et al., 2021).

FTIR spectroscopy has been employed to characterize functional groups present in carotenoids from various sources (Kushwaha et al., 2014; Quijano-Ortega et al., 2020). As shown in Table 3, spectral regions near 1650 cm^{-1}, 1400 cm^{-1}, and 2900–2800 cm^{-1} have also been reported in studies involving bacterial carotenoids (Kushwaha et al., 2014; Lóránd et al., 2022; Udensi et al., 2022), corresponding to bands observed in the *K. palustris* extract in this study.

Table 3 Main bands observed in the FTIR spectrum of the carotenoid broth produced by *K. palustris*.

Número de onda (cm^{-1})	Possível atribuição	Grupo funcional

3441	Estiramento O-H	Hidroxila
2925	Estiramento C-H assimétrico	CH_2/CH_3 alifático
2854	Estiramento C-H simétrico	CH_2/CH_3 alifático
1744	Estiramento C=O	Carbonila
1637	Estiramento C=C	Ligações duplas conjugadas
1465	Deformação angular C-H	CH_2/CH_3
1378	Deformação simétrica C-H	CH_3
1189	Estiramento C-O	Álcool/éster
721	Rocking C-H	Cadeia alifática longa

Source: Prepared by the authors based on Amin et al. (2015) and Sharma and Goshal (2020).

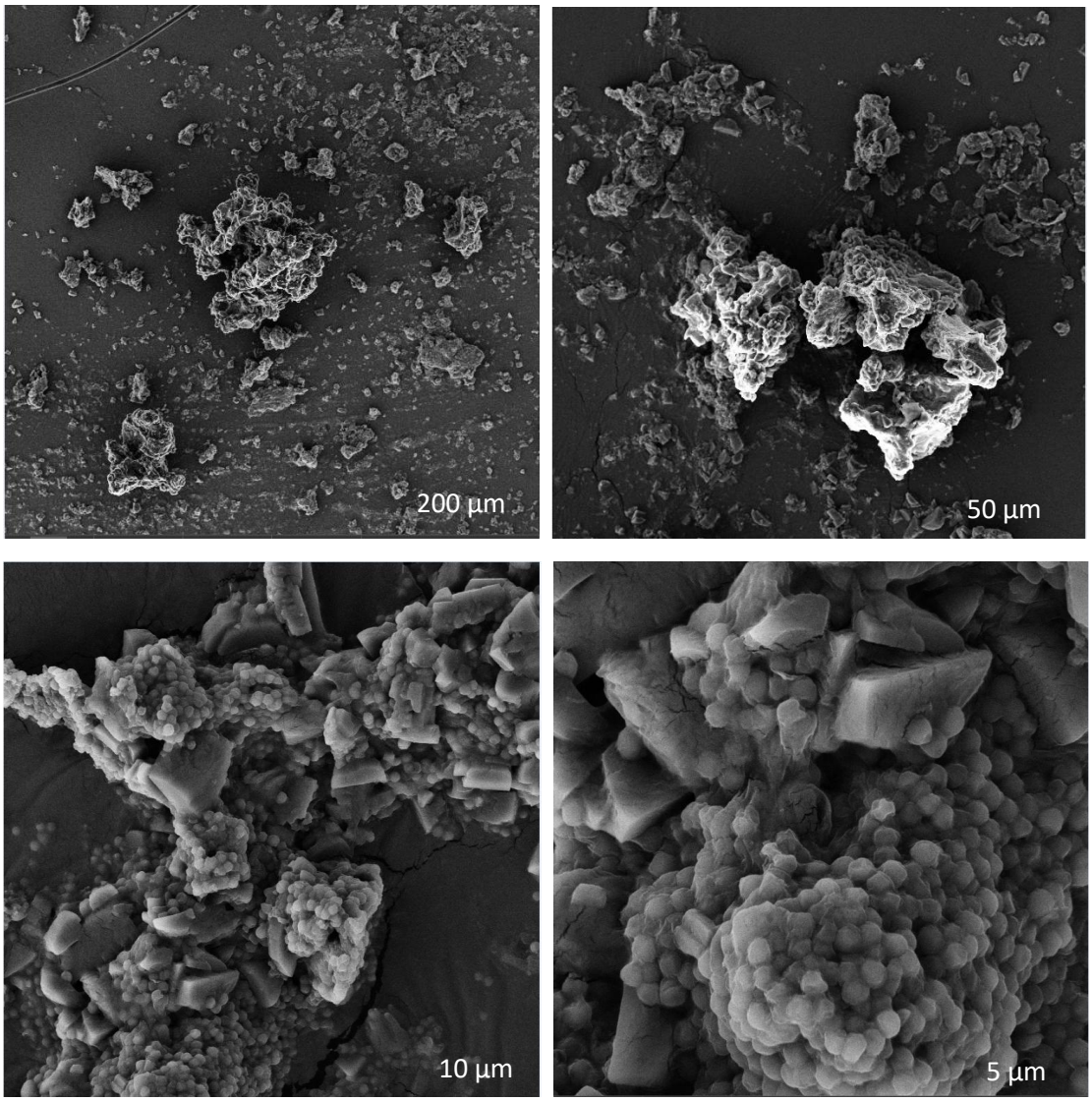
Considering the set of spectroscopic results, the observed profile exhibits characteristics consistent with carotenoids. The conjugated double-bond system, evidenced by bands associated with C=C vibrations, corresponds to a structural feature common to carotenoids, while bands related to oxygenated groups are consistent with compounds of the xanthophyll class. Furthermore, the UV-Vis absorption maxima matched the spectral profile described in the literature for sarcinaxanthin and its derivatives—C50 carotenoids found in pigmented Gram-positive bacteria (Netzer et al., 2010). Thus, the results obtained via UV-Vis and FTIR indicate consistency with the presence of sarcinaxanthin, although they are insufficient for its conclusive identification.

Morphological and elemental characterization of the extract by SEM/EDS

To complement the characterization of the *K. palustris* freeze-dried extract, SEM was employed to evaluate its surface morphology, while EDS was used to characterize the elemental composition of the analyzed regions. These analyses served as complementary characterization techniques for the extract rather than for the structural identification of the carotenoids.

The SEM micrographs (Figure 5) allowed for the observation of the solid material's surface morphology at various magnifications, revealing the formation of irregular aggregates, likely resulting from the freeze-drying process. This tendency toward aggregation characterizes the organization of the extract in the solid state but does not allow for the determination of the chemical identity of the compounds present.

Figure 5 Micrograph obtained via Scanning Electron Microscopy (SEM) showing the surface morphology of the carotenoid extract at 200 μm, 50 μm, 10 μm, and 5 μm.

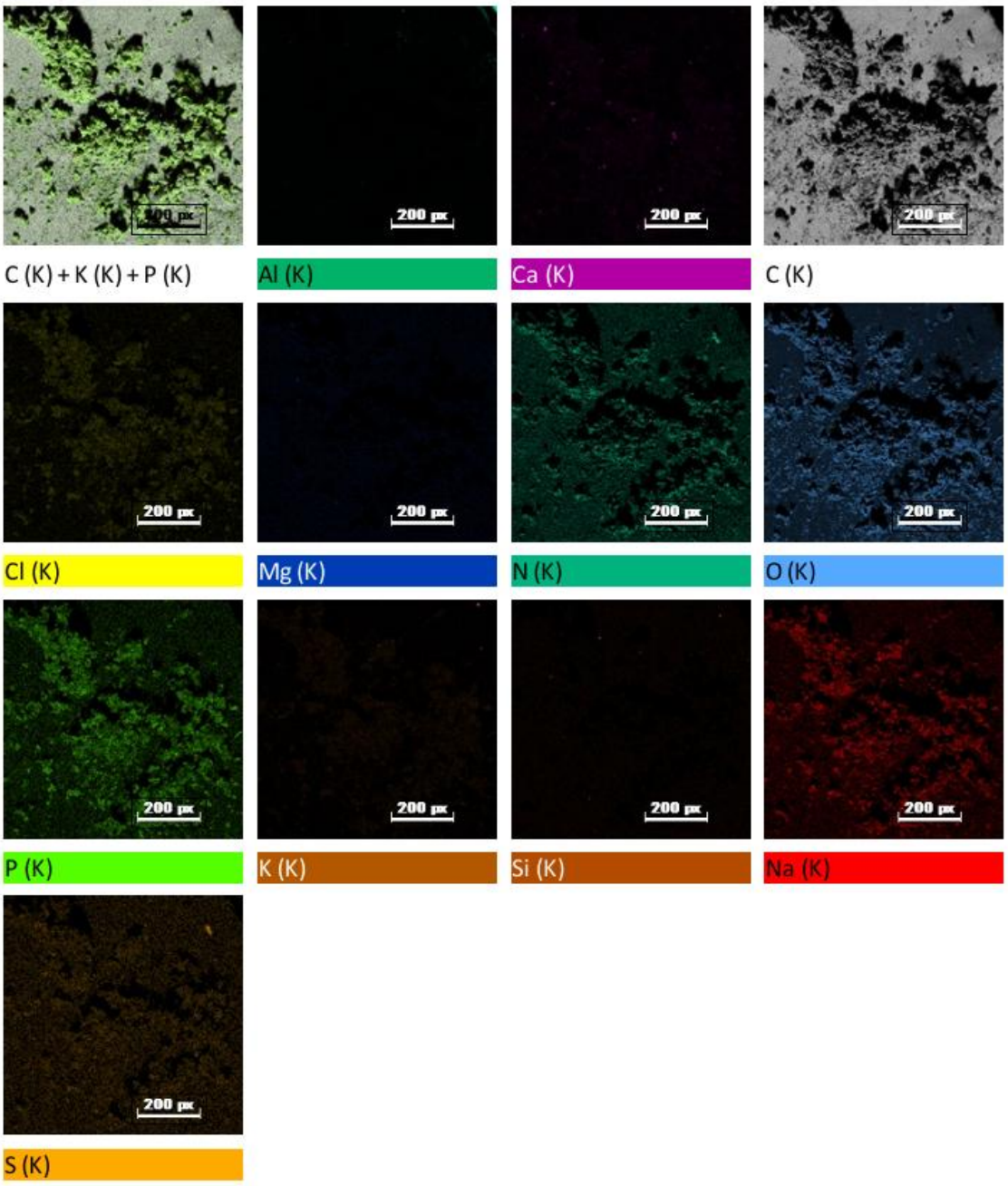


In a study conducted by Contreras et al. (2025), SEM was used to assess the biological effect of carotenoids produced by *Arthrobacter* sp. LAPM80, demonstrating that cells rich in these pigments exhibited minimal morphological changes following UV-B radiation exposure compared to less pigmented cells.

Thus, while that study indirectly highlighted the photoprotective role of carotenoids at the cellular level, the present work utilized SEM to characterize the morphological organization of the isolated pigment, revealing its tendency to aggregate in the solid state. Taken together, these results are consistent with the recognized structural stability of carotenoids and their capacity to act as photoprotective agents.

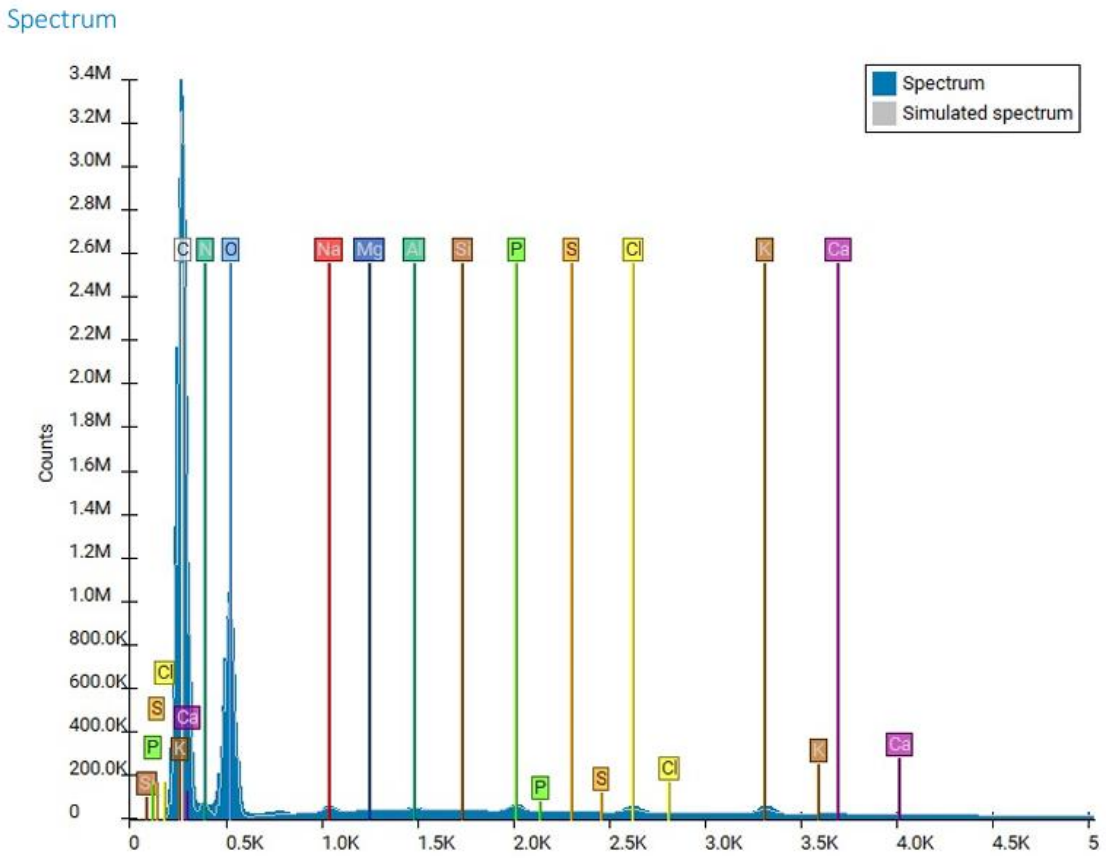
EDS analysis was performed to complement SEM, aiming to characterize the elements present in the analyzed regions of the lyophilized extract. Elemental maps revealed a broad distribution of carbon (C) and oxygen (O), as well as the presence of nitrogen (N), sodium (Na), phosphorus (P), potassium (K), calcium (Ca), silicon (Si), and sulfur (S)—the latter elements showing lower intensity and a more localized distribution.

Figure 6 EDS results showing the elemental composition of the analyzed sample.



The EDS spectrum shown in Figure 7 corroborated the elemental mapping results, displaying higher relative intensities for the carbon and oxygen signals. The predominance of these elements is consistent with the organic nature of the sample but does not constitute specific evidence for the identification of carotenoids, given that carbon and oxygen are present in various organic components.

Fig. 7 EDS spectrum of the sample, highlighting the peaks corresponding to the chemical elements present. The “K” notation in the spectrum refers to electronic transitions from the K-shell of the analyzed atoms.



The predominance of carbon and oxygen is consistent with the organic nature of the analyzed extract. It should be noted that hydrogen, although present in organic compounds, is not detected by EDS due to the technique's limitations regarding low-atomic-number elements (Goldstein et al., 2018). The other elements detected in minor proportions may be associated with the sample's complex matrix, including biomass components, residues from the culture medium, or materials from sample preparation steps. Since the culture medium was not subjected to elemental analysis separately, it was not possible to determine the specific origin of these elements.

In vitro photoprotective activity

SPF indicates a sunscreen's ability to reduce the harmful effects of ultraviolet radiation on the skin (Sundar Lingakumar, 2023). This property is directly related to the UV radiation absorption capacity of the compounds present in the formulation. In this regard, high absorption in the 290–320 nm range suggests that carotenoids have the potential to increase SPF when incorporated into photoprotective formulations (Suryawanshi et al., 2015).

Supporting this photoprotective potential, the crude carotenoid extract from *K. palustris*, at a concentration of 100 mg/L, exhibited an SPF of 41.21. This result is significantly higher than the minimum value established by Resolution RDC Nº. 30 of June 1, 2012, which mandates a minimum SPF of 6.0 for products classified as sunscreens (Brazil, 2012).

Thus, when compared to studies available in the literature, the SPF value obtained in this work also stands out. Mendes-Silva et al. (2021) and Schmidt et al. (2021), using carotenoids produced in synthetic medium, reported SPF values of 9.36 ± 0.52 for isolate FT-7.22 and 4.26 ± 0.83 for isolate FT5.10, respectively. Similarly, Kusmita et al. (2023), using carotenoids produced by *Virgibacillus* sp. in synthetic medium, obtained an SPF of 6.04. Furthermore, these authors classified the extracts according to their photoprotective potential—as shown in Table 4—with the extract produced in the present study falling into the ultra-protection category.

Table 4 Sun protection categories according to SPF.

SPF	Protection Category
2 – 4	Minimum
4 – 6	Moderate
6 – 8	Extra
8 – 15	Maximum
≥ 15	Ultra

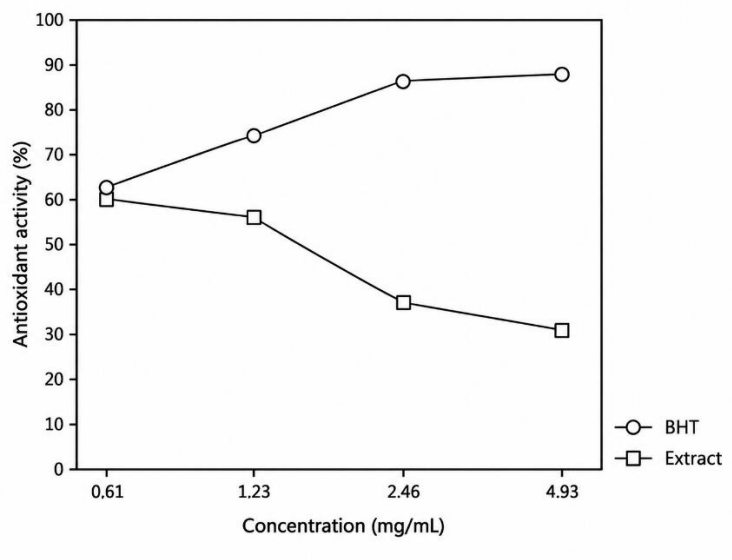
Given this, the medium containing TPJ, whey, and glucose enabled the production of an extract by *K. palustris* with high photoprotective activity. However, considering the extract's complex composition and the potential presence of compounds derived from the culture medium, the observed SPF cannot be attributed solely to the carotenoids produced by the bacterium.

The combined effects of microbial pigments and other photoprotective components were evaluated by Suryawanshi et al. (2015), who added prodigiosin and violacein—produced by *Serratia marcescens* and *Chromobacterium violaceum*, respectively—to commercial photoprotective formulations and botanical extracts. The addition of these pigments increased the formulations' SPF, demonstrating that the combination of different components can alter photoprotective activity. Unlike that study, the present work did not evaluate the medium components and the carotenoids produced by *K. palustris* in isolation. Therefore, although the extract exhibited a high SPF, it is not possible to establish the occurrence of synergism between the bacterial carotenoids and the compounds derived from TPJ. Future studies evaluating these components both separately and in combination are needed to determine their respective contributions to photoprotective activity.

In vitro antioxidant activity

The results of the in vitro antioxidant activity of the *K. palustris* carotenoid extract and the BHT standard, determined using the β-carotene/linoleic acid system, are presented in Figure 8.

Fig. 8 Antioxidant activity curves of the carotenoid extract and the BHT standard at different concentrations, determined by the β-carotene/linoleic acid system.



The extract demonstrated antioxidant activity ranging from 31.82% to 61.36%, with the highest values observed at the lowest concentrations evaluated. Unlike the BHT standard, which showed a progressive increase in antioxidant activity alongside rising concentration, the extract did not exhibit dose-dependent behavior.

For the carotenoid extract, behavior inversely proportional to concentration was observed. The lowest dose tested (0.61 mg/mL) exhibited the greatest system-protective capacity, achieving 61.36% inhibition of β-carotene bleaching. As the extract concentration increased to 1.23 mg/mL, 2.46 mg/mL, and 4.93 mg/mL, protective efficacy progressively decreased to 56.82%, 38.64%, and 31.82%, respectively. In contrast, the synthetic standard BHT exhibited classic dose-dependent behavior within the same working range. The standard's antioxidant activity increased in a stepwise manner, starting at 63.64% (at 0.61 mg/mL) and reaching peak protection at 4.93 mg/mL (88.63%).

The comparison between the curves demonstrates that, while the loss of linearity in the BHT standard stems from the physical saturation of the micellar interface, the deviation observed in the crude extract is governed by chemical co-oxidation mechanisms. These findings highlight that the carotenoid extract under study exhibits excellent antioxidant efficiency (exceeding 61%) when applied at low concentrations, indicating a superior capacity to protect the β-carotene/linoleic acid system against oxidation.

The results obtained differ from those reported by Mendes-Silva et al. (2021), who, using the β-carotene/linoleic acid system, observed an increase in the antioxidant activity of carotenoids produced by *K. palustris* as the concentration increased. In the present study, the opposite behavior was observed, with a reduction in antioxidant activity at the highest concentrations evaluated. The reduction in antioxidant activity observed at higher concentrations may indicate a loss of the extract's antioxidant efficiency, and further studies are needed to determine whether pro-oxidant behavior occurs.

Another possible explanation for the behavior observed in this antioxidant activity relates to the nature of the extract used. Although sarcinaxanthin was identified as the major carotenoid, the material is a crude extract obtained from the cultivation of *K. palustris* in a medium containing tangerine peel broth, whey, and glucose. Thus, the matrix may contain other carotenoids and compounds derived from bacterial metabolism and the culture medium that could interfere with the antioxidant activity of sarcinaxanthin, especially at higher concentrations.

In this context, it is plausible that the absence of a purification step limited the expression of the major carotenoid's antioxidant activity due to interactions between matrix constituents. Consequently, studies comparing the crude extract with sarcinaxanthin-enriched or purified fractions could clarify the influence of these compounds on the observed antioxidant activity.

Conclusions

The results obtained demonstrate that *K. palustris* was able to grow and produce carotenoids in a medium formulated with alternative agro-industrial substrates—specifically tangerine peel juice (TPJ) and whey—supplemented with glucose. These results highlight the potential of using these substrates as nutrient sources for bacterial cultivation and carotenoid production.

Under the conditions evaluated, *K. palustris* produced carotenoids with a profile consistent with sarcinaxanthin, and the resulting extract exhibited antioxidant and photoprotective properties, underscoring the biotechnological interest in these pigments.

The experimental design demonstrated that glucose and TPJ significantly influenced carotenoid production and SPF, with the magnitude of the effect being greater for TPJ. The selected condition was applied to a scale-up process, in which *K. palustris* maintained its carotenoid production capacity, indicating the potential for using the formulated medium in bioprocess development.

The extract exhibited high photoprotective activity, achieving an SPF of 41.21—classified within the "very high protection" range. Antioxidant activity, determined via the β-carotene/linoleic acid system, ranged from 31.82% to 61.36%, highlighting the extract's functional potential, although the response did not show a dose-dependent pattern at the concentrations evaluated.

Overall, the results demonstrate that cultivating *K. palustris* using alternative agro-industrial substrates is a promising strategy for valorizing these materials and obtaining biomolecules with photoprotective and antioxidant properties. As a scientific contribution, this study expands knowledge regarding *K. palustris* cultivation by demonstrating the feasibility of using TPJ and whey in combination as alternative substrates and showing that carotenoid production is maintained upon scale-up, thereby contributing to the development of production strategies linked to the utilization of agro-industrial waste.

However, industrial application still requires further advances regarding the structural confirmation of the produced carotenoids, process optimization and

control in bioreactors, standardization of the waste-based medium composition, pigment recovery and purification, and the assessment of stability, safety, and efficacy. Furthermore, studies on productivity, techno-economic feasibility, and performance in formulations are necessary to determine the process's competitiveness and applicability at an industrial scale.

Funding

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