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Chemical, Microbial, and Sensory Characterization of Juice Prepared Using Carotenoid Pigment Produced by Locally Isolated *Rhodotorula mucilaginosa* Yeast

Manal S. Mahdi, Ali H. Abdulwahhab, Ali N. Abdul-Ghaffar
Deportment of Food Science, College of Agriculture, Tikrit University, Iraq

***Correspondence: manalsaleh@tu.edu.iq**

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ABSTRACT

This study investigated the extraction of microbial carotenoids from *Rhodotorula mucilaginosa* and evaluated their potential application as natural colorants in juice formulations. The antibacterial activity of the bio produced β-carotene demonstrated a clear concentration-dependent effect, with inhibition zones reaching 21.67 mm for *E. coli*, 18.0 mm for *Staphylococcus aureus*, and 20.0 mm for *Salmonella spp.* at 50 mg/mL. Sensory evaluation revealed no significant differences among carotenoid-fortified juice, commercial juice, and natural juice across all attributes, indicating high consumer acceptability. Chemical analysis showed a significant increase in total acidity in carotenoid-fortified and natural juices (0.18–0.19), while commercial juice recorded the lowest value (0.13). Mineral content (K, Ca, Na) and pH exhibited no significant variation among treatments, whereas total soluble solids were highest in commercial juice (0.45 g/100 mL). Microbiological assessment confirmed the absence of *E. coli*, yeasts, and molds in all samples, with low total bacterial counts, reflecting adequate microbial quality. Overall, the findings demonstrate that microbial β-carotene is a promising natural pigment with functional antibacterial activity and suitable technological performance for use in juice production.

التقييم الكيميائي والميكروبي والحسي للعصير المحضَّر باستخدام صبغة الكاروتينويد المنتجة من خميرة *Rhodotorula mucilaginosa* المعزولة محلياً

منال صالح مهدي، علي حسن عبدالوهاب، علي نزار عبدالغفار
قسم علوم الأغذية، كلية الزراعة، جامعة تكريت، العراق

الخلاصة

بحثت هذه الدراسة استخلاص الكاروتينويدات الميكروبية من خميرة *Rhodotorula mucilaginosa* وتقييم إمكانية تطبيقها كملونات طبيعية في تصنيع العصائر. وأظهرت التراكيز المختلفة من البيتا كاروتين المنتج حيويًا فعالية مضادة للميكروبات، إذ بلغت أقطار التثبيط 21.67 مم لبكتريا *E.coli*، و18.0 مم لبكتريا *Staphylococcus aureus*، و20.0 مم لبكتريا *Salmonella Spp.* عند تركيز 50 ملغم/مل. كما أظهر التقييم الحسي عدم وجود فروق معنوية بين العصير المدعَّم بالكاروتينويد والعصير التجاري والعصير الطبيعي في جميع الصفات المدروسة، مما يشير إلى قبول حسي مرتفع. وبيّنت نتائج التحاليل الكيميائية حدوث زيادة معنوية في الحموضة الكلية في العصير المدعَّم والعصير الطبيعي (0.18–0.19)، في حين سجّل العصير التجاري أقل قيمة (0.13). ولم تُسجَّل فروق معنوية في محتوى المعادن (البوتاسيوم، الكالسيوم، الصوديوم) أو قيم الأس الهيدروجيني بين المعاملات، بينما سجّل العصير التجاري أعلى قيمة للمواد الصلبة الذائبة (0.45 غ/100 مل). وأكّد التقييم الميكروبي خلو جميع العينات من *E. coli* والخمائر والعفن، مع تسجيل أعداد بكتيرية كلية منخفضة، وهو ما يعكس جودة ميكروبية مناسبة. بصورة عامة، تُظهر النتائج أن بيتا كاروتين الميكروبي يُعدّ صبغة طبيعية واعدة تمتلك نشاطًا مضادًا للبكتريا وأداءً تقنيًا مناسبًا يُمكّن من استخدامها في صناعة العصائر.

الكلمات المفتاحية: بيتا كاروتين، كاروتينويدات، *Rhodotorula mucilaginosa*، ملونات طبيعية للعصائر.

INTRODUCTION

Color is a critical sensory attribute influencing consumer perception, acceptance, and overall quality evaluation of food products. The increasing demand for natural and safe food ingredients has shifted global attention toward replacing synthetic colorants with natural alternatives due to safety concerns and regulatory restrictions. Microbial pigments have gained prominence in recent years because they offer stable production, high yields, and independence from seasonal variations, making them particularly suitable for industrial applications (Sen, Barrow, & Deshmukh, 2019). Among microbial pigments, carotenoids represent one of the most valuable classes due to their vibrant coloration, antioxidant properties, and functional significance in food systems.

Yeasts belonging to the genus *Rhodotorula* are well recognized for their ability to produce carotenoids such as β-carotene, torularhodin, and torulene. Their capability to grow on low-cost substrates and tolerate diverse environmental conditions makes them promising candidates for biotechnological pigment production (Rodrigues *et al.,* 2019). Recent studies have demonstrated that *Rhodotorula mucilaginosa* strains can be optimized through controlled fermentation strategies, nutrient modulation, or experimental design approaches to enhance pigment yield, especially when utilizing agro-industrial byproducts or alternative culture media (Garcia-Cortes *et al.,* 2021; Torres-Álvarez *et al.,* 2022). Furthermore, carotenoid biosynthesis pathways in *Rhodotorula* spp. have been increasingly elucidated, enabling better understanding of metabolic regulation and genetic mechanisms that influence pigment formation (Tang *et al.,* 2019).

Despite advances in carotenoid production from *Rhodotorula*, limited research has explored the practical application of these microbial pigments in food formulations such as fruit juices, particularly regarding their impact on chemical attributes, microbial stability, and sensory acceptability. Most available studies focus on improving pigment productivity, while fewer investigate the functional, antimicrobial, or sensory implications of incorporating yeast-derived carotenoids into real food matrices. Additionally, the

utilization of pigments extracted from locally isolated strains remains underreported, even though local adaptation may enhance growth, stability, and pigment quality.

Therefore, the present study aims to evaluate the chemical, microbial, and sensory properties of juice formulated using carotenoid pigment extracted from a locally isolated *Rhodotorula mucilaginosa* yeast. The study also assesses the antimicrobial activity of the pigment to determine its potential role not only as a natural colorant but also as a contributor to microbial control in juice products. This integrated assessment provides new insights into the feasibility and practical benefits of microbial carotenoids as sustainable colorants for the food and beverage industry.

MATERIALS AND METHODS

Sterilization:

Glassware and instruments requiring dry sterilization were treated in a hot air oven at 180 °C for 2 h. Inoculating loops and other metallic tools were sterilized by direct flaming using a Bunsen burner. Culture media and aqueous solutions requiring moist heat sterilization were autoclaved at 121 °C and 15 psi for 15 min, following the standard protocol described by Al-Maamouri and Al-Maamouri (2016).

Preparation of culture media:

The culture media used in this study were prepared by dissolving the appropriate amounts of dehydrated media in distilled water, according to the manufacturer's instructions. Each medium was brought to a boil to ensure complete dissolution and subsequently sterilized at 121 °C for 15 min. After cooling to approximately 50 °C, the media were poured into sterile Petri dishes and allowed to solidify. The sterility of the prepared plates was verified by incubating them at 30 °C for 24 h, as described by Atlas (2004).

Source of yeast isolate:

The yeast isolate *Rhodotorula mucilaginosa* BA61 was obtained from the laboratories of the College of Agriculture, Tikrit University. This isolate had been previously identified and characterized in earlier work conducted in the Department of Food Sciences.

Preservation of yeast isolates:

The yeast isolate was maintained on Yeast–Malt Extract Agar (YMA) slants prepared in test tubes. The isolate was streaked onto the slants using an inoculating loop and incubated at 30 °C for 24 h. After visible growth was established, the culture surface was overlaid with sterile liquid paraffin to ensure long-term preservation and stored at 5 °C, following the method described by Bhosale (2001).

Carotenoids Extraction:

Carotenoids were extracted following a modified version of the method described by Park et al. (2005). A 50-mL aliquot of the fermentation broth was centrifuged at 3,500 rpm for 15 min, and the resulting pellet was washed repeatedly with distilled water until

the washings became colorless. The cleaned biomass was then mixed with equal volumes of acetone and hexane. Cell disruption was performed using an ultrasonic processor at 59 kHz and 40 °C for 30 min to ensure efficient intracellular pigment release.

The mixture was left at room temperature overnight to enhance solvent penetration and maximize extraction efficiency. On the following day, the pigment-containing solvent was separated by filtration through Whitman filter paper. The extraction was repeated several times until the biomass turned completely colorless, indicating full pigment recovery.

The combined filtrates were transferred to a separating funnel, and the hexane phase was purified by adding 15% (w/v) sodium chloride solution to facilitate phase separation. The clarified hexane layer was collected, and its absorbance was measured at 450 nm using a UV–Vis spectrophotometer to quantify total carotenoids.

The inhibitory effect of bioproduced beta-carotene:

The antimicrobial activity of the bioproduced β-carotene pigment was evaluated against selected foodborne pathogenic bacteria, including *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*. These bacterial strains were obtained as pure cultures and reactivated prior to testing. The inhibitory effect was assessed using the agar well-diffusion method as described by Holt et al. (1994).

Mueller–Hinton agar plates were inoculated by evenly spreading a bacterial suspension adjusted to 0.5 McFarland standard using a sterile cotton swab. Wells of 6 mm diameter were aseptically punched into the agar using a sterile cork borer. Different concentrations of the β-carotene extract (10, 20, 30, 40, and 50 mg/mL) were dispensed into the wells. The plates were incubated at 37 °C for 24 h, after which the inhibition zones surrounding the wells were measured to determine the antibacterial effectiveness of the pigment.

Juice Manufacturing:

The juice formulation procedure was performed following the method described by Kovács et al. (2011), with modifications to incorporate the microbial β-carotene pigment.

Materials

Sucrose, distilled or purified water, citric acid, microbial β-carotene pigment, and orange flavoring.

Procedure

1. A sugar solution was prepared by dissolving 650 g/L of sucrose in 1 L of distilled water and heating the mixture to 65 °C until complete dissolution was achieved, resulting in a final concentration of approximately 65%, depending on general acceptability standards.
2. Citric acid (5–10 g/L) was added, with the amount adjusted proportionally to the sugar concentration.
3. The microbial β-carotene pigment was added gradually to the juice base and mixed thoroughly to ensure uniform color distribution and prevent sedimentation. The

developed color was compared with both commercial and natural juice samples to achieve an acceptable visual match.

4. The juice was then cooled to refrigeration temperature, and 2–3 mL/L of orange flavoring was added.
5. A sensory evaluation was subsequently conducted to compare the prepared juice with commercial and natural juice samples.

Sensory Evaluation of Processed Juice:

A sensory evaluation was carried out to assess the juice formulated with microbial β-carotene pigment in comparison with commercial and natural juice samples. The evaluation was performed by faculty members and students from the Department of Food Science using the prepared sensory evaluation form. A hedonic scale was employed, in which scores of 0–4 indicated "poor," 5–6 "average," 7 "good," 8 "very good," and scores above 8 represented "excellent."

Panelists evaluated the juice samples for color, taste, flavor, texture, and overall acceptability. All assessments were conducted under controlled laboratory conditions to ensure consistency and minimize bias.

Chemical composition of the juice:

Chemical tests:

Estimation of total acidity:

Total titratable acidity was determined using the titration method described by Abdullah (2004). Juice samples were titrated with 1 N sodium hydroxide using phenolphthalein as an indicator, with citric acid considered the predominant organic acid in the juice matrix.

Determination of Heavy Metals and Mineral Content:

Heavy metals and selected minerals were quantified using an Atomic Absorption Spectrophotometer, model E (LCO), following the procedures outlined by A.O.A.C. (2008). The analyses were conducted at the Department of Chemical Engineering, Tikrit University.

Samples were first subjected to dry ashing. Ash weight was calculated using the formula:

Ash weight (g) = (Weight of sample + crucible before incineration) – (Weight of crucible + ash after incineration)

Ash percentage (%) = (Ash weight ÷ Sample weight) × 100

To determine the concentrations of potassium, calcium, and sodium, the ash residue was dissolved in 5 mL of 5% nitric acid and mixed thoroughly. The solution was then filtered, and the clear filtrate was analyzed using atomic absorption spectroscopy. Each mineral concentration was measured directly from the liquid sample aspirated into the instrument.

Physical Tests:

1. Total Dissolved Solids (TDS): Total dissolved solids in the juice samples were measured using a handheld refractometer at 19 °C. The readings were expressed as °Brix.
2. pH Measurement: The pH of the juice samples was measured using a calibrated digital pH meter, following the procedure described by Jamaludin et al. (2016).
Microbial Tests:
1. Total Bacterial Count: The total viable bacterial count was determined according to the method outlined by APHA (1984). Serial dilutions were plated on Nutrient Agar, and the plates were incubated at 37 °C for 24–48 h before colony enumeration.
2. Total Yeast and Mold Count: Yeast and mold counts were obtained by plating appropriate dilutions on Malt Extract Agar. After incubation, fungal colonies were identified using the taxonomic key described by Winn et al. (2006).
3. *Escherichia coli*: Coliform bacteria, including *E. coli*, were enumerated using the pour plate method as described by Amelia et al. (2020). The inoculated plates were incubated at 37 °C for 48 h prior to counting.

Statistical Analysis

All experiments were conducted in triplicate, and data were expressed as **mean ± standard deviation (SD)**. Statistical analyses were performed using **IBM SPSS Statistics version 26** (IBM Corp., Armonk, NY, USA). Differences among treatments were evaluated using **one-way analysis of variance (ANOVA)**. When ANOVA indicated significant effects, **Duncan's multiple range test** was applied for post-hoc comparison of means. Statistical significance was established at $\mathbf{p \leq 0.05}$. Prior to analysis, data were examined for **normality (Shapiro–Wilk test)** and **homogeneity of variances (Levene's test)** to ensure compliance with ANOVA assumptions.

RESULTS AND DISCUSSION

Table 1 presents the antibacterial activity of bioproduced β-carotene against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella spp.*, which represent common foodborne pathogens. At the lowest concentration (10 mg/mL), inhibition zones of 13.0 mm for *E. coli* and *S. aureus*, and 14.67 mm for *Salmonella* were observed, with no significant differences ($p \leq 0.05$). Increasing pigment concentrations from 20 to 50 mg/mL resulted in a significant ($p \leq 0.05$) and concentration-dependent enhancement in antibacterial activity, reaching maximum values of 21.67 mm for *E. coli*, 18.0 mm for *S. aureus*, and 20.0 mm for *Salmonella* at 50 mg/mL.

Recent studies have demonstrated similar trends, showing that carotenoids produced by *Rhodotorula* species exhibit notable antibacterial activity across multiple foodborne pathogens (Iqbal *et al.*, 2024; Kim *et al.*, 2021; Zhao *et al.*, 2024). The lipophilic properties of β-carotene allow the pigment to integrate into bacterial cell membranes, altering membrane fluidity and permeability. This mechanism is supported by contemporary reports indicating that microbial carotenoids disrupt membrane integrity,

induce oxidative stress, and promote leakage of intracellular components (Saubenova *et al.,* 2024; Ochoa-Viñals *et al.,* 2024). Such effects help explain the stronger response observed in the Gram-positive *S. aureus*, whose thick peptidoglycan-rich wall is more susceptible to lipophilic compounds than the outer membrane characteristic of Gram-negative bacteria.

The non-linear inhibitory pattern observed for *Salmonella* at intermediate concentrations may reflect strain-specific oxidative defense mechanisms, as documented in recent ecological studies of carotenogenic yeasts under stress conditions (Akhtyamova and Sattarova, 2024). These variations indicate that interactions between pigments and bacterial cells are influenced by both concentration and bacterial physiology.

Overall, these findings highlight the potential application of microbial β-carotene as a natural colorant with additional antimicrobial functionality in food systems, particularly at higher concentrations.

Table 1. Inhibitory activity of β-carotene pigment against selected foodborne bacteria at different concentrations.

	Damping diameter (mm)				
	Concentration				
Bacteria	**10 mg/Ml**	**20 mg/mL**	**30 mg/mL**	**40 mg/mL**	**50 mg/mL**
E. coli	13.00 ± 0.58 A a	21.00 ± 0.58 A a	21.00 ± 0.58 A a	22.67 ± 0.33 A a	21.67 ± 0.88 A a
Staphylococcus aureus	13.00 ± 1.73 A b	15.00 ± 1.15 B b	14.33 ± 0.88 B b	17.67 ± 0.88 B b	18.00 ± 0.58 B b
***Salmonella* spp.**	14.67 ± 0.33 A ab	17.67 ± 0.88 B ab	17.33 ± 1.20 B ab	21.00 ± 0.58 A ab	20.00 ± 0.00 A ab

Different uppercase letters (A, B) within the same row indicate significant differences ($p \leq 0.05$) between concentrations.
Different lowercase letters (a, b, ab) within the same column indicate significant differences ($p \leq 0.05$) among bacterial species.
Values are expressed as mean ± standard deviation.

Table 2 summarizes the sensory attributes of juices prepared with microbial β-carotene (T2), compared with commercial juice (T1) and natural orange juice (T3). The results indicated no significant differences ($p \leq 0.05$) among treatments for color, taste, flavor, density, texture, and overall acceptability, although numerical variations were observed. T1 and T2 demonstrated comparable results across most attributes, likely due to formulation standardization in sweeteners, acidity, and flavoring agents commonly used in manufactured juices. The sensory stability observed in T2 suggests that microbial β-carotene can function as a natural colorant without negatively affecting consumer acceptance. These findings are consistent with recent reports indicating that microbial carotenoids enhance color uniformity and contribute to flavor stability due to their antioxidant activity (Molina *et al.,* 2023).

Natural orange juice (T3) showed lower scores in taste, texture, and overall acceptability. This pattern is commonly reported in juices without stabilizers or homogenizers, where natural variability in raw fruit composition results in differences in viscosity, pulp distribution, and flavor intensity (Watcharawipas, 2022; Raita *et al.,* 2023). This does not indicate inferior nutritional quality; rather, it reflects consumer preference for standardized commercial beverages with enhanced mouth feel and uniformity. Overall, the data support the potential of microbial β-carotene as a natural pigment suitable for use in beverage formulations without compromising key sensory properties.

Table 2. Sensory evaluation characteristics of juices with microbial carotenoids compared with commercial and natural juices

	Attribute					
Treatment	**Color**	**Taste**	**Flavor**	**Density**	**Texture**	**Overall Acceptability**
T1	8.37 ± 0.32 A	7.30 ± 0.52 A	7.68 ± 0.29 a	7.12 ± 0.27 A	7.78 ± 0.23 A	7.82 ± 0.41 A
T2	7.17 ± 0.55 A	8.07 ± 0.44 A	6.78 ± 0.49 a	6.34 ± 0.16 A	6.48 ± 0.09 B	6.91 ± 0.07 Ab
T3	6.99 ± 0.28 A	6.32 ± 0.64 A	6.63 ± 0.63 a	6.81 ± 0.37 A	6.80 ± 0.25 B	6.52 ± 0.29 B

Different uppercase letters (A, B) within a column indicate significant differences ($p \leq 0.05$).
Different lowercase letters (a, b) indicate significant differences ($p \leq 0.05$).
Sensory attributes were evaluated using a hedonic scale (0–10).
T1 = Commercial juice,T2 = Juice formulated with microbial β-carotene,T3 = Natural orange juice.

Table 3 presents the total acidity, expressed as % citric acid, for commercial juice (T1), juice enriched with microbial β-carotene (T2), and natural orange juice (T3). Total acidity showed a significant increase ($p \leq 0.05$) in T2 and T3, reaching 0.18% and 0.19%, respectively, compared with the commercial product T1 (0.13%). The higher acidity in T3 reflects the natural content of organic acids—primarily citric acid—commonly found in fresh citrus juices. This is consistent with recent studies reporting natural acidity levels between 0.15–0.25% in unprocessed orange juice (Frontiers in Sustainable Food Systems, 2023; Raita *et al.,* 2023).

The relatively elevated acidity in T2, which contains microbial β-carotene, may be associated with formulation adjustments during preparation. Natural pigments often require balancing of acidity to stabilize color intensity and maintain sensory attributes, a relationship noted by Araya-Cloutier et al. (2023) and Sinha et al. (2022), who reported that bioactive pigments can interact with organic acids and influence acid–base equilibrium during formulation.

In contrast, T1 exhibited significantly lower acidity. This pattern is commonly observed in industrial juices, where acidity regulators and flavor balancers are added to

enhance palatability and reduce sourness perceived by consumers. Commercial processing may also dilute natural acids through blending, thermal treatment, or the addition of sweeteners, a trend supported by recent industrial juice quality assessments (De La Torre *et al.,* 2021; Kim and Lee, 2023).

Overall, the results indicate that microbial β-carotene can be incorporated into juice formulations without reducing natural acidity, and that T2 maintains acidity levels comparable to natural juice, enhancing its potential as a clean-label functional beverage.

Table 3. Total acidity (% citric acid) in commercial, natural, and β-carotene–enriched juices.

Treatment	Total Acidity (% citric acid)
T1	0.13 ± 0.01 B
T2	0.18 ± 0.01 A
T3	0.19 ± 0.003 A

Different uppercase letters indicate significant differences ($p \leq 0.05$).

T1 = Commercial juice,T2 = Juice formulated with microbial β-carotene,T3 = Natural orange juice.

Potassium (K), calcium (Ca), sodium (Na) in commercial juice, microbial β-carotene and natural orange juice The concentration of potassium- K, calcium - Ca, and sodium Na in the commercial, juice T1juice fortified with microbial β- carotene T2and standard orange juices is presented in Table 4. All measured minerals did not differ significantly ($p > 0.05$) between treatments.` The variation in potassium content was ranged from 21.50 to 22.23 mg/L and T3 showed the highest value (Table A2) due to the natural difference among fresh citrus fruits. The differences in calcium (24.03–25.06 mg/L) and sodium (1.43–1.70 mg/L) were also not significant. These results indicate that both industrial process and addition of microbial carotenoids did not affect the content of essential minerals. Recently, it has been reported that gentle pasteurization and typical juice processing conditions do not impact mineral stability since heat-stable mineral elements are resistant to pignment incorporation or pH property change (Liu *et al.,* 2023); Guleria et al. "Natural juices (T3)" might contain slight differences in potassium and sodium, resulting from the cultivar variety and soil characteristics as well as maturation process according to compositional modern surveys of citric beverages already publishedValores de referencia (USDA, 2021; Moulehi *et al.,* 2022). The similarity between T1 and T2 in the mineral levels suggested that microbial β-carotene is either not bound with ionic elements or did not disrupt the mineral balance, similar to other studies (Carvalho *et al.,* 2018; Detmann *et al.,* 2024) where carotenoid additives only operate as pigment without affecting the mineral profile. According to this logic, the lack of significant differences between treatments indicates that β-carotene did not modify juice nutritional mineral composition..

Table 4. Mineral concentrations (mg/L) in commercial, natural, and β-carotene–enriched juices

	Element		
Treatment	**Potassium (mg/L)**	**Calcium (mg/L)**	**Sodium (mg/L)**
T1	21.50 ± 0.47 A	25.06 ± 0.02 A	1.43 ± 0.26 A
T2	21.83 ± 0.33 A	24.13 ± 0.09 A	1.50 ± 0.06 A
T3	22.23 ± 0.52 A	24.03 ± 0.88 A	1.70 ± 0.15 A

Different uppercase letters within a column indicate significant differences at ($p \leq 0.05$).
T1 = Commercial juice,T2 = Juice formulated with microbial β-carotene,T3 = Natural orange juice.

Some of the physicochemical characteristics of commercial juice (T1), microbial β-carotene-fortified juice (T2) and natural orange juice (T3) are presented in Table 5. Total soluble solids (g/100 mL) significantly differed among treatments (Table 2) and their mean difference was significant ($p \leq 0.05$); T1 gave the highest value ($p \leq 0.05$) and others like T2(0.42 g/100 mL), andT3(0.37 g/100 mL). This is in accordance with basically a relationship between sweeteners and stabilizer/thickeners but different concentrations that remain fairly constant of these groups during fermentation. These trends are also observed in commercial formulations which, including stabilizers, sweetener and thickeners result in higher total solids as evidenced recently through beverage formulation (Zhang *et al.,* 2022; Martins et al., 2023). The low value of T3 is consistent with the natural composition of fresh juice unadulterated by industry and agrees qualitatively with a behavior expected for minimally processed citrus juices. Ash % Ash content did not vary among treatments indicating that juices had similar mineral contents. This, thus shows that the fruit source of the raw materials or processing conditions has no influence on inorganic compounds as reported in a recent study carried out on citrus beverage processing (Muleke *et al.,* 2021). Total sugars were significantly higher in T2 and T3 than those of T1 with that of T3 recorded the highest (8.05 g/100 mL). The natural juices in the main have much greater proportions of these intrinsic sugars (sucrose, glucose and fructose) than revealed by these recent composition analyses of fresh OJ (Raita *et al.,* 2023; Bagheri *et al.,* 2022). T1 had relatively lower sugar compared to both T2 and CB, a reason for this could be dilution or substitution of sugar or even balancing effect of acid regulator in order to attain an acceptable standardized flavor quality widely documented in commercial juice production (Awolu *et al.,* 2010).

No differences in ascorbic acid content were found between treatments, and the levels for all samples were approximately 0.03 g/100 mL. This stability suggests that mild heat treatment and oxygen were probably applied in processing, as vitamin C is highly unstable to heat or oxygen. Some studies have reported that temperature-controlled processing is useful to perform good ascorbic acid retention in yam flour (Alvarenga *et al.,* 2022; Choi *et al.,* 2023).

Also, pH values did not show significant differences, being that T3 was the greatest (4.00), resulting in natural juice slightly acid. The lowest pH (3.43) in T2 might be that there was a change in organic acid during production. These results are also supported by those reported that pH is primarily determined by individual acids but not total acidity (Nuzzi *et al.,* 2022). In general, these results indicate that the addition of microbial β-carotene has no influence on the most important physicochemical parameters and the natural juice (T3) preserves its typical chemical profile without any industrial supplementation..

Table 5. Physicochemical composition of commercial, β-carotene–enriched, and natural juices.

	Attribute				
Treatment	**Total Solids (g/100 mL)**	**Ash (g/100 mL)**	**Total Sugars (g/100 mL)**	**Ascorbic Acid (g/100 mL)**	**pH**
T1	0.45 ± 0.02 A	0.29 ± 0.01 A	7.65 ± 0.02 B	0.03 ± 0.00 a	3.73 ± 0.05 A
T2	0.42 ± 0.01 Ab	0.26 ± 0.01 A	7.98 ± 0.02 A	0.03 ± 0.003 a	3.43 ± 0.34 A
T3	0.37 ± 0.03 B	0.27 ± 0.02 A	8.05 ± 0.08 A	0.03 ± 0.003 a	4.00 ± 0.20 A

Different uppercase letters within a column indicate significant differences ($p \leq 0.05$).
Different lowercase letters indicate no significant differences ($p > 0.05$).
T1 = Commercial juice; T2 = Juice formulated with microbial β-carotene; T3 = Natural orange juice.

Table 6 presents the microbial content of commercial juice (T1), β-carotene–enriched juice (T2), and natural orange juice (T3). No significant differences ($p > 0.05$) were observed among treatments regarding total bacterial count, *E. coli*, or yeasts and molds. Total bacterial counts were low in T1 and T2 (3.00 CFU/mL), while T3 recorded a slightly higher count (5.33 CFU/mL), although still within acceptable microbiological limits for fresh juice products. The low microbial counts in T1 and T2 reflect the effectiveness of industrial processing, including pasteurization and hygienic packaging, which are known to significantly reduce microbial load in beverage products. Recent studies confirm that mild heat treatments and aseptic filling are efficient in lowering total aerobic counts without compromising product quality (Silva *et al.,* 2022; Gama *et al.,* 2023). The similarity between T1 and T2 indicates that the inclusion of microbial β-carotene did not negatively affect microbial stability, supporting findings that natural pigments do not promote microbial growth and may even provide mild inhibitory effects due to their antioxidant nature (Fasolin *et al.,* 2021; De Andrade *et al.,* 2023). *E. coli* was completely absent in all treatments, confirming good manufacturing practices (GMP), absence of fecal contamination, and overall process hygiene. The absence of yeasts and molds in all samples further reflects effective processing, adequate packaging, and proper storage conditions. Modern evaluations of juice safety emphasize the importance of controlling airborne contaminants, sugar-handling steps, and closure integrity to prevent fungal contamination (Moussa *et al.,* 2021; Shori, 2023). The slightly higher bacterial count in T3 is typical of fresh, non-processed juice, which lacks thermal treatment and is more susceptible to environmental contamination during extraction or handling. Natural juices typically harbor

small numbers of aerobic bacteria derived from fruit surfaces, equipment, or handling stages (Tango *et al.,* 2022). However, the levels observed in T3 remained low and did not indicate deteriorative or pathogenic contamination.

Overall, the results confirm that enriching juice with microbial β-carotene does not compromise microbial safety and that all products meet acceptable standards for total bacterial count and absence of indicator organisms.

Table 6. Microbial content (CFU/mL) of commercial, β-carotene–enriched, and natural juices

Treatment	Total Bacterial Count (CFU/mL)	*E. coli* (CFU/mL)	Yeasts and Molds (CFU/mL)
T1	3.00 ± 1.00 A	0.00 ± 0.00 A	0.00 ± 0.00 a
T2	3.00 ± 0.58 A	0.00 ± 0.00 A	0.00 ± 0.00 a
T3	5.33 ± 1.20 A	0.00 ± 0.00 A	0.00 ± 0.00 a

Similar lowercase or uppercase letters within a column indicate no significant differences ($p > 0.05$).
T1 = Commercial juice; T2 = Juice formulated with microbial β-carotene; T3 = Natural orange juice.

CONCLUSION

This study demonstrated that microbial β-carotene extracted from *Rhodotorula mucilaginosa* can be successfully incorporated into fruit juice formulations without adversely affecting physicochemical, sensory, or microbiological quality. The enriched juice (T2) showed acceptable acidity, mineral stability, ascorbic acid retention, and sensory attributes comparable to both commercial and natural juices. The carotenoid pigment also exhibited notable antimicrobial activity, particularly at higher concentrations, highlighting its potential as a natural functional additive. Overall, the findings support the feasibility of using microbially produced carotenoids as safe, stable, and effective natural colorants in juice manufacturing.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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