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Optimized Production Astaxanthin on Sugarcane Molasses by *X. dendrorhous* in 3-LBioreactor

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ABSTRACT

Astaxanthin, a high-value xanthophyll carotenoid with potent antioxidant properties, has garnered significant interest in nutraceutical and pharmaceutical industries. *Xanthophyllomyces dendrorhous* is a promising microbial source for astaxanthin biosynthesis; however, its industrial application is often constrained by suboptimal yields and high production costs. In this study, central composite design with 20 run was used to optimize process parameters of glucose concentration (7–13 g/L), yeast extract (1.5–4.5 g/L), and initial pH (5.5–7.5) in shake flask cultures. The optimized conditions (7 g/L glucose, 1.5 g/L yeast extract, pH 5.5) led to a 1.7-fold increase in astaxanthin production, with maximum biomass yields of 14.20 g/L (wet) and 4.139 g/L (dry). The regression model showed high predictive accuracy (R^2 = 0.9741; F = 41.78). To further reduce production costs, alternative carbon sources, including sodium acetate, sodium citrate, and sugarcane molasses (10 g/L), were evaluated. sugarcane molasses supported pigment yields comparable to glucose-based media while substantially lowering media costs. Among four extraction methods tested, DMSO-based extraction demonstrated the highest recovery efficiency. The extracted pigment exhibited 96% DPPH radical scavenging activity, indicating strong antioxidant potential. Results were validated in a 3-L bioreactor (at 22–25 °C, 120 rpm, 4 L/min aeration, and initial pH 5.5), confirming process scalability. These findings demonstrate that integrating medium optimization and low-cost substrates represents a viable and scalable strategy for enhanced microbial astaxanthin production in biotechnological applications.

What is "already known":	• Optimized condition achieved at optimum glucose, yeast extract, and pH, boosting astaxanthin yield by 1.7-fold, with 14.20 g/L and 4.139 g/L (dry) biomass. • Sugarcane molasses (10 g/L) matched glucose-based media performance, significantly reducing production costs. • DMSO-based extraction achieved the highest carotenoid recovery compared to other methods. • Extracted astaxanthin exhibited 96% DPPH scavenging activity, surpassing vitamin C (85%), vitamin E (78%), and β-carotene (68%). • Optimized conditions validated in a 3-L bioreactor, confirming scalability for industrial applications.
What this article adds:	• Achieves groundbreaking 22% cost reductions and 50% energy savings (0.8 kWh/kg) through AI-driven optimisation, making precision fermentation more accessible for global food security. • Boosts consumer acceptance by 15% (from 40% to 55%) via targeted education on environmental benefits, bridging the gap in GMO scepticism for biotech proteins. • Unlocks waste valorisation potential, slashing production costs by 20% with fruit waste, enhancing the circular economy in precision fermentation for a greener future. • Scalability analysis, forecasting 15,000 metric tons of protein by 2026 using 100,000 L bioreactors, aligning with 1.5°C climate goals in sustainable food systems.

1. Introduction

Carotenoids are a diverse group of naturally occurring pigments synthesized by plants, algae, fungi, and certain bacteria. These lipophilic compounds play crucial biological roles as antioxidants, provitamin A precursors, immune enhancers, and photoprotectants [1]. Among over 700 identified carotenoids, astaxanthin (a red-orange keto-carotenoid belonging to the xanthophyll class) has received growing attention due to its extraordinary antioxidant activity, estimated to be 10 times stronger than β-carotene and up to 100 times greater than α-tocopherol [2,3]. This bioactive compound has demonstrated potential therapeutic applications in oxidative stress-related disorders, including cardiovascular diseases, neurodegeneration, cancer, and skin aging [4,5].

The commercial demand for astaxanthin continues to rise, driven by its applications in nutraceuticals, pharmaceuticals, aquaculture feed, and cosmetics [6]. Currently, the market is dominated by chemically synthesized astaxanthin, which is cost-effective but associated with several drawbacks, such as lower bioavailability, potential toxicity, and consumer preference for natural products [7]. Consequently, there has been increasing interest in sustainable biological production using microalgae (e.g., *Haematococcus pluvialis*) and yeasts (e.g., *Xanthophyllomyces dendrorhous*) as natural producers of astaxanthin [8, 9].

X. dendrorhous offers distinct advantages over microalgae, including faster growth rates, simpler cultivation requirements, and the potential for genetic and metabolic engineering to enhance carotenoid biosynthesis [10]. However, large-scale production remains economically challenging due to the high cost of conventional growth media, particularly carbon and nitrogen sources like glucose and yeast extract [11]. This has prompted a shift toward evaluating low-cost, renewable substrates, such as agro-industrial by-products (molasses, glycerol, acetate, and citrate), which not only reduce production costs but also support circular bioeconomy strategies [12].

To improve pigment yield while minimizing costs, researchers have applied various optimization strategies, including statistical design of experiments. Among these, Response Surface Methodology (RSM) has emerged as a robust tool to model complex interactions among multiple process variables and identify optimal culture conditions with fewer experimental runs [13]. While significant advancements have been made in optimizing fermentation parameters for maximum astaxanthin production, relatively few studies have comprehensively addressed the downstream processes, such as extraction efficiency and the functional characterization of the extracted pigment. The method of pigment extraction is crucial for industrial applications, as it impacts yield, purity, and antioxidant properties of astaxanthin. Traditional organic solvent-based methods may result in variable recovery and raise concerns regarding environmental and health safety. Therefore, exploring and comparing environmentally friendly, high-efficiency extraction techniques is essential [14,15]. Furthermore, evaluating the antioxidant activity of extracted astaxanthin using reliable assays like 2,2-diphenyl-1-picrylhydrazyl (DPPH) helps validate its biofunctional potential for therapeutic and food-grade applications [16].

In this study, we aimed to enhance astaxanthin production from *X. dendrorhous* by integrating medium optimization using RSM and employing cost-effective carbon sources (acetate, citrate, and molasses). We further assessed the impact of different extraction methods on pigment recovery and evaluated the antioxidant potential of the extracted astaxanthin using the DPPH radical scavenging assay. Finally, the scalability of the optimized fermentation strategy was validated in shake-flask and bioreactor systems to evaluate its industrial feasibility.

2. Materials and Methods

2.1. Microorganism and Culture Conditions

The yeast strain *Xanthophyllomyces dendrorhous* IBRC-M 30167 was obtained from the Iranian Biological Resource Center (IBRC, Tehran, Iran). The strain was routinely maintained on Yeast and Mold (YM) agar medium composed of glucose, yeast extract, malt extract, and soybean peptone (Quelab, Canada). Reagents including glucose, sodium acetate, sodium citrate, sugarcane molasses, and ammonium phosphate were purchased from Iranian Ovin Biotech (Tehran, Iran), and agar was obtained from Merck (Germany).

2.2. Activation and Pre-culture

The strain was activated on YM agar plates incubated at 25 °C for 48 h. For long-term storage, a cryopreservation mixture containing YM medium, glycerol, and skim milk was used. Samples were initially kept at −20 °C for 1 h and then transferred to −30 °C. For seed culture, 50 mL of liquid YM medium was inoculated with the activated yeast and incubated at 25 °C on a rotary shaker (120 rpm) for 24 h [17].

2.3. Shake Flask Fermentation

2.3.1. Standard YM Cultivation

Initial shake flask fermentations were conducted in Erlenmeyer flasks using standard YM medium (10 g/L glucose, 3 g/L yeast extract, 3 g/L malt extract, and 5 g/L soybean peptone), adjusted to pH 5.5. Cultures were incubated at 22 °C with agitation at 120 rpm for 120 h. Biomass, total carotenoids, and astaxanthin concentrations were measured at regular intervals.

2.3.2. Optimization via Response Surface Methodology (RSM)

Optimization of carbon and nitrogen sources and pH was performed using Response Surface Methodology (RSM) based on a Central Composite Design (CCD) in Design Expert software (Version 11). The model evaluated glucose (7–13 g/L), yeast extract (1.5–4.5 g/L), and pH (5.5–7.5) to identify optimal conditions for biomass and pigment production. Model accuracy was assessed using analysis of variance (ANOVA) and R^2 coefficients.

Table 1. Independent variables and their levels used in the central composite design.

Factor	Symbol	Unit	Low	High	-alpha	+alpha
Glucose concentration	A	g/L	7	13	5.5	15.5
Yeast extract	B	g/L	1.5	4.5	0.75	5.25
Initial pH	C	—	5.5	7.5	5	8

2.3.3. Supplementation with Alternative Carbon Sources

To explore cost-effective substrates, sodium acetate and sodium citrate were each added at 1.0, 2.0, and 3.0 g/L either at inoculation or 24 h post-inoculation. Cultures were based on the optimized YM medium (pH 5.5), incubated at 22 °C and 120 rpm for 120 h. Final biomass and pigment production were quantified [18, 19].

2.3.4. Cultivation in Molasses-Based Medium

Industrial simulation was performed using a molasses-based medium (10 g/L sugarcane molasses, 3 g/L malt extract, and 1.1 g/L ammonium phosphate). Flasks (100 mL and 250 mL) were incubated at 22 °C, 120 rpm for 120 h at pH 5.5. Biomass and pigment yields were compared with other media.

2.4. Bioreactor Cultivation

Batch fermentation was carried out in a 3 L stirred-tank bioreactor (working volume: 2.7 L). The vessel and accessories were autoclaved at 121 °C for 40 min. Separately, 30 g glucose was dissolved in 300 mL distilled water, sterilized at 110 °C for 10 min, and added aseptically after cooling. Two media were tested: (i) standard YM medium and (ii) optimized molasses-based medium, both inoculated with 300 mL of pre-culture. Fermentations ran at 22–25 °C, 120 rpm, 4 L/min aeration, and initial pH 5.5–5.6. Samples were taken every 12 h to measure OD600 and biomass (wet/dry) [20].

2.5. Carotenoid Extraction and Quantification

2.5.1. Biomass Preparation

Biomass was harvested by centrifugation at 10,845 × g for 10 min. Wet cells were stored at 4 °C (short-term) or frozen at −20 °C and then freeze-dried for 24 h prior to pigment extraction. For each extraction method, 50 mg of lyophilized biomass was used [21].

2.5.2. Evaluation of Extraction Methods

Four protocols (two mechanical and two chemical) were compared:

Glass Bead Milling: Biomass suspended in 5 mL methanol with glass beads, vortexed twice for 5 min, centrifuged (9000 rpm, 15 min), supernatant collected [22].

Ultrasonication: Biomass in methanol (1:10 w/v), stirred for 1 h, sonicated (100 W, 10 min, ice bath), centrifuged at 9000 rpm [23].

Acid Hydrolysis: Biomass treated with 3 mL of 3 M HCl at 100 °C for 3 min, cooled, washed, then extracted with 5 mL acetone [24].

DMSO Extraction: 50 mg dry biomass in 5 mL DMSO, incubated at 60 °C for 24 h in the dark, vortexed, centrifuged, and pigments extracted into 10 mL acetone. This method consistently produced the highest carotenoid yield and was used for all experiment [17].

2.5.3. Quantification of Astaxanthin

Astaxanthin content in the extracts was measured spectrophotometrically at 480 nm. Concentration (µg/g dry biomass) was calculated using the equation 1:

$$\text{Astaxanthin}(\mu g/g\ \text{dryYeast}) = \frac{\text{A480 nm} \times \text{VV}}{16 \times \text{WW}} \times 100 \quad \text{Eq. (1)}$$

Where A480 is the absorbance, VV is the solvent volume (mL), and WW is the weight of the dry biomass (g). Results were validated by HPLC analysis using a C18 column and external astaxanthin standard [25].

2.6. Antioxidant Activity Assay

The antioxidant activity of extracted pigments was evaluated using the DPPH (2,2-diphenyl-1-picryl-hydrazyl) assay. One milligram of each sample (vitamins A, C, E, K, antioxidant powder, and commercial astaxanthin) was dissolved in 1 mL methanol. After mixing with 100 µL DPPH reagent and incubating at room temperature for 30 min, absorbance was measured at 517 nm. Methanol served as the blank, and distilled water was used as a negative control. The radical scavenging activity was calculated by Equation 2::

$$\text{DPPH scavenging activity}(\%) = \frac{A_{control} - (A_{sample} - A_{blank})}{A_{control}} \times 100 \quad \text{Eq. (2)}$$

Where $A_{control}, A_{sample}$, and A_{blank} represent the absorbance of the DPPH solution in methanol, the absorbance of the astaxanthin solution with DPPH, and the absorbance of the astaxanthin solution without DPPH, respectively [26].

3. Results and Discussion

3.1. Optimization of Cultivation Conditions Using Response Surface Methodology (RSM)

In this study, Response Surface Methodology (RSM) was employed to optimize the cultivation conditions for *Xanthophyllomyces dendrorhous* to enhance biomass and astaxanthin production. A Central Composite Design (CCD) was utilized to evaluate the effects of glucose concentration, yeast extract concentration, and pH on the responses. A total of 20 experiments were conducted, and the data were analyzed using Design-Expert software.

Table 2. The results of dry biomass weight measurements and absorbance at a wavelength of 630 nanometers for the experiments designed using the central composite method under various cultivation conditions

STD	Glucose Concentration (g/l)	Yeast Extract Concentration (g/l)	pH	Response1 Absorbance at 630 nm	Dry Biomass Weight (g/l)
1	7.0	1.5	5.5	0.51	5
2	13.0	1.5	5.5	0.71	5.5
3	7.0	4.5	5.5	0.541	3.5
4	13.0	4.5	5.5	0.059	4
5	7.0	1.5	7.5	0.055	3.5
6	13.0	1.5	7.5	0.587	4.5
7	7.0	4.5	7.5	0.554	5
8	13.0	4.5	7.5	0.486	3.5
9	5.5	3.0	6.5	0.495	3.5
10	14.5	3.0	6.5	0.567	0.5
11	10.0	0.75	6.5	0.54	3
12	10.0	5.25	6.5	0.45	1
13	10.0	3.0	5.0	0.545	4
14	10.0	3.0	8.0	0.468	3.5
15	10.0	3.0	6.5	0.545	3
16	10.0	3.0	6.5	0.561	2.5
17	10.0	3.0	6.5	0.508	2.5
18	10.0	3.0	6.5	0.552	3
19	10.0	3.0	6.5	0.524	2.5
20	10.0	3.0	6.5	0.552	3

Among the 20 experimental runs designed using Central Composite Design (CCD), STD number 2 yielded the highest dry biomass (5.5 g/L). However, run number 1, which achieved slightly lower biomass (5 g/L), was considered superior due to its lower glucose concentration (7 g/L), which makes it more

cost-effective and metabolically efficient. Notably, this run also resulted in a significantly higher astaxanthin yield, suggesting that carbon limitation may favor pigment biosynthesis in *X. dendrorhous*.

The data indicate that higher glucose concentrations did not proportionally increase astaxanthin production, possibly due to metabolic diversion toward cell proliferation rather than secondary metabolite synthesis. Therefore, despite not having the absolute highest biomass, the cultivation condition of run 1 was selected as optimal for downstream applications, considering both yield efficiency and pigment productivity.

Table 3. *Analysis of variance for the quadratic model evaluating the effects of glucose, yeast extract, and ph on biomass and astaxanthin production by Xanthophyllomyces dendrorhous*

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.4585	9	0.0509	41.78	< 0.0001	significant
A-Glu	0.0067	1	0.0067	5.52	0.0407	
B-Yeast	0.0102	1	0.0102	8.36	0.0161	
C-pH	0.0051	1	0.0051	4.22	0.0671	
AB	0.2054	1	0.2054	168.49	< 0.0001	
AC	0.0696	1	0.0696	57.05	< 0.0001	
BC	0.1295	1	0.1295	106.24	< 0.0001	
A^2	0.0049	1	0.0049	4.05	0.0717	
B^2	0.0148	1	0.0148	12.14	0.0059	
C^2	0.0111	1	0.0111	9.09	0.0130	
Residual	0.0122	10	0.0012			
Lack of Fit	0.0102	5	0.0020	5.00	0.0510	not significant
Pure Error	0.0020	5	0.0004			
Cor Total	0.4707	19				

The effects of glucose concentration (A), yeast extract concentration (B), and pH (C) on the biomass and astaxanthin production by *Xanthophyllomyces dendrorhous* were statistically evaluated using a Central Composite Design (CCD) and analyzed through a second-order polynomial model.

The analysis of variance (ANOVA) revealed that the model was highly significant ($p< 0.0001$), with an F-value of 41.78, confirming its suitability for predicting the response (Table 4). The interaction terms AB, AC, and BC were especially influential, showing F-values of 168.49, 57.05, and 106.24 respectively ($p < 0.0001$ for all), highlighting the synergistic effects between the variables. Among the linear terms, glucose (A) and yeast extract (B) had statistically significant effects ($p = 0.0407$ and 0.0161 respectively), while pH (C) was borderline significant ($p = 0.0671$). The quadratic terms B^2 and C^2 were also statistically significant ($p < 0.05$), suggesting curvature in the response surface, particularly with respect to yeast extract and pH.

Table 4. *Statistical Summary of the Second-Order Regression Model for Predicting Biomass and Astaxanthin Yield*

Std. Dev.	0.0349	R^2	0.9741
Mean	0.4905	Adjusted R^2	0.9508
C.V. %	7.12	Predicted R^2	0.8362
		Adeq Precision	26.8097

Data of Table 4 demonstrated a strong goodness-of-fit with a high coefficient of determination ($R^2 = 0.9741$), indicating that 97.41% of the variation in biomass and astaxanthin production could be explained by the model. The adjusted R^2 (0.9508) and predicted R^2 (0.8362) values further confirmed the

model's robustness and predictive accuracy, with a reasonable agreement between the experimental and predicted data. The low standard deviation (0.0349) and coefficient of variation (CV = 7.12%) indicate high precision and reproducibility of the experimental results. Moreover, the Adequate Precision value of 26.81, which greatly exceeds the recommended threshold of 4.0, signifies a strong signal-to-noise ratio and suggests that the model has sufficient discriminatory capability to navigate the design space effectively.

To visualize the interactive effects of glucose (A) and yeast extract (B) on biomass and astaxanthin production at varying pH levels, contour and 3D surface plots were generated based on the fitted response surface model.

Contour plots (Figure 1A–C) depict the two-dimensional interaction between glucose and yeast extract concentrations at constant pH levels of 5.5, 6.5, and 7.5, respectively. These plots reveal the non-linear nature of the interactions, with elliptical contour lines indicating significant second-order effects. At pH 5.5 (Figure 1A), an increase in both glucose and yeast extract concentrations led to a higher predicted biomass, suggesting a synergistic interaction. Conversely, at pH 6.5 (Figure 1B), glucose had a more dominant influence, while further increases in yeast extract did not proportionally enhance biomass yield. At pH 7.5 (Figure 1C), both factors showed diminished effectiveness, indicating suboptimal conditions for growth.

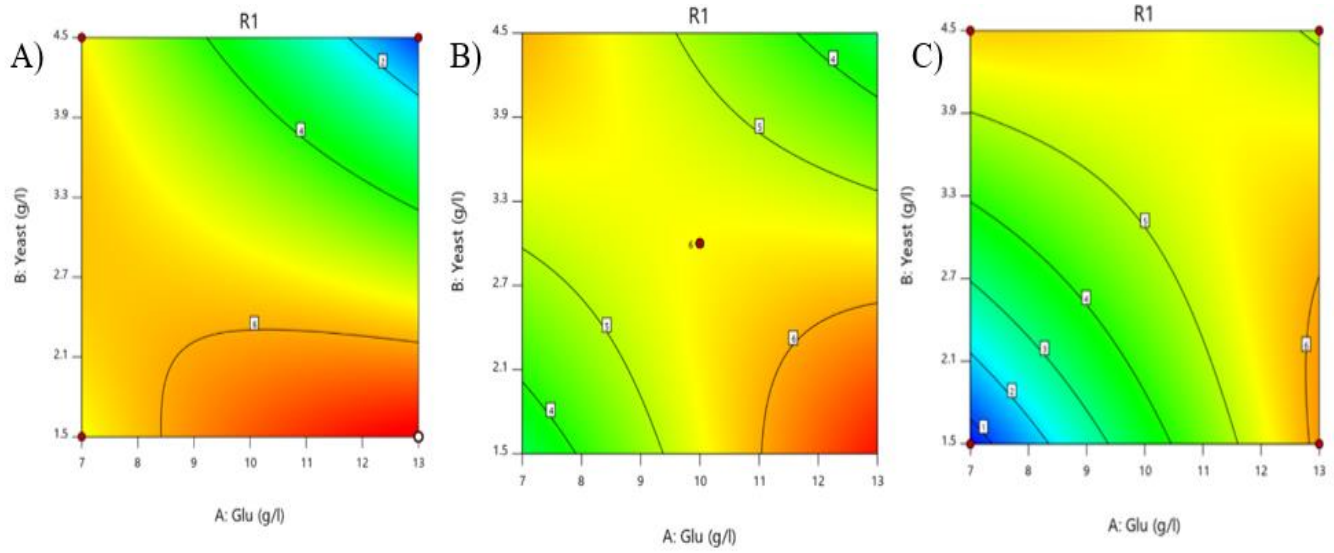

Figure 1. Three contour models illustrating the effects of variables a and B at a constant pH of A) 5.5 B) 6.5 C) 7.5

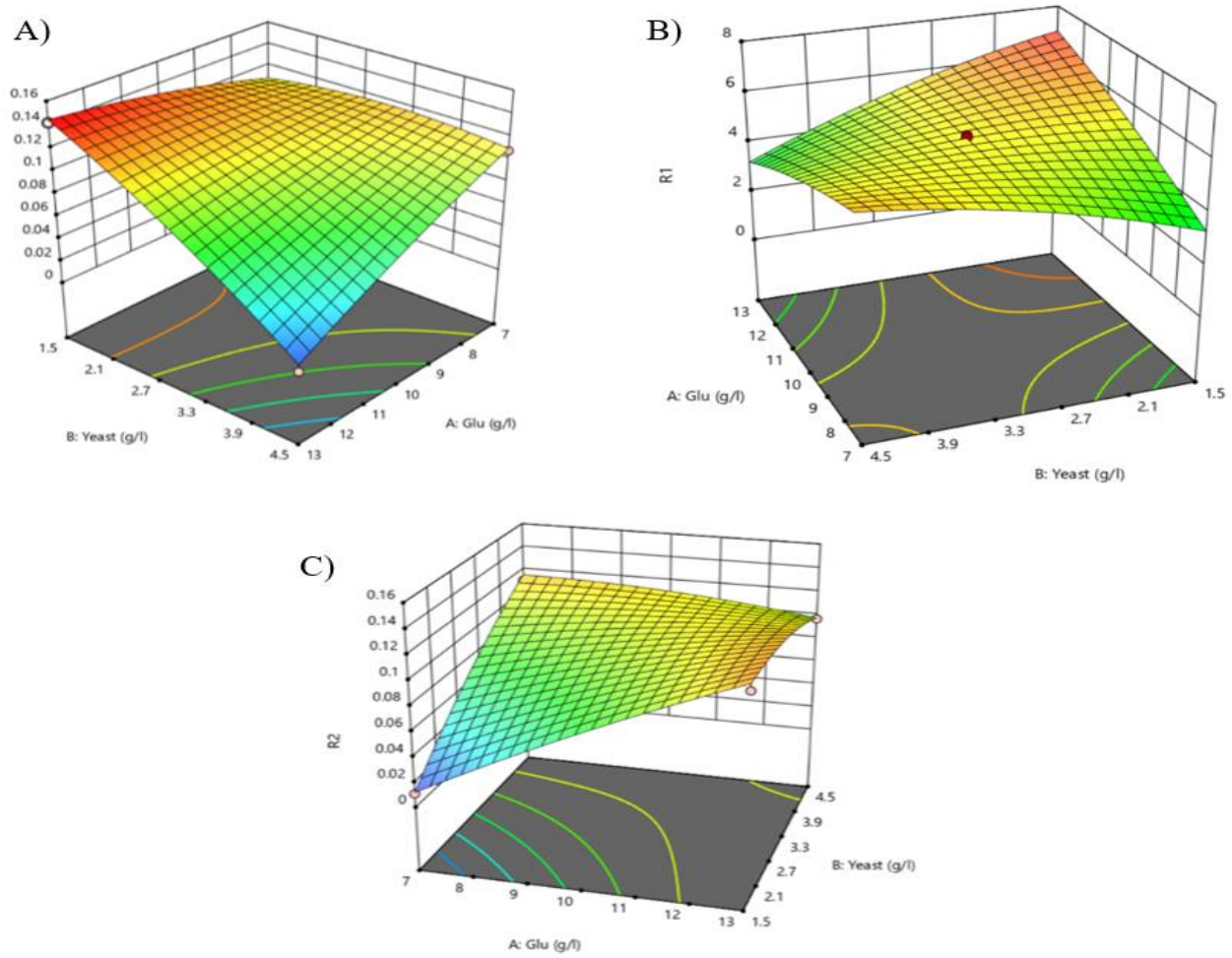

Figure 2. Three dimensional models illustrating the effects of variables A and B at a constant pH of A) 5.5 B) 6.5 C) 7.5

3D surface plots (Figure 2A–C) further illustrate these interactions by offering a topographical view of the response surface under the same pH conditions. The three-dimensional perspective enables identification of local maxima and saddle points, highlighting the curvature of the response. The peak response was observed at intermediate levels of glucose and yeast extract, particularly under slightly acidic conditions (pH 5.5), which aligns with previous findings on the optimal physiological range of *Xanthophyllomyces dendrorhous*.

These graphical models serve as a powerful tool to complement the regression analysis, enabling intuitive understanding of the optimal operational region within the design space. Based on visual and statistical interpretation, the optimal region was identified around 7 g/L glucose and 1.5 g/L yeast extract at pH 5.5, corroborating the results derived from numerical optimization.

3.2. Effect of Sodium Acetate and Sodium Citrate on Yeast Growth

The addition of sodium acetate and sodium citrate as supplementary carbon sources significantly impacted yeast growth. When added at the beginning of cultivation, 3 g/L sodium acetate and 3 g/L sodium citrate resulted in the highest dry biomass of 4.88 g/L and 6.04 g/L, respectively. However, using these compounds as sole carbon sources did not support yeast growth, indicating the necessity of glucose for optimal cultivation.

Table 5. Dry biomass weight of samples containing sodium citrate and sodium acetate at the beginning of growth

Concentration	Dry Biomass Weight (g/L)	Carotenoid(µg/g)
1 g/L Sodium Citrate	4.12± 0.01	16.234
2 g/L Sodium Citrate	2.92± 0.01	8.520
3 g/L Sodium Citrate	6.04± 0.01	15.386
1 g/L Sodium Acetate	3.48 ± 0.01	14.666
2 g/L Sodium Acetate	4.52 ± 0.01	11.238
3 g/L Sodium Acetate	4.88 ± 0.01	6.190

Table 5 summarizes the dry biomass and carotenoid content of *Xanthophyllomyces dendrorhous* cultures supplemented with varying concentrations of sodium citrate and sodium acetate during the early growth phase. Among the citrate-treated samples, the culture with 3 g/L sodium citrate exhibited the highest biomass yield (6.04 ± 0.01 g/L), indicating enhanced cellular growth at this concentration. Interestingly, the highest carotenoid accumulation (16.234 µg/g) was observed at the lowest citrate concentration (1 g/L), suggesting that excess citrate may stimulate growth but not pigment synthesis.

In contrast, sodium acetate displayed a concentration-dependent increase in biomass, peaking at 3 g/L (4.88 ± 0.01 g/L). However, carotenoid production declined with increasing acetate concentration, with the maximum observed at 1 g/L (14.666 µg/g). These results suggest a divergent impact of organic acid salts on biomass and carotenoid biosynthesis, possibly due to differences in their metabolic assimilation or influence on cellular redox status.

3.3. Biomass Production in Bioreactor Using YM and Molasses-Based Media

To evaluate biomass production under controlled conditions, batch fermentations were conducted in a 1-liter bioreactor using two different media: the standard YM medium and a molasses-based medium selected based on prior Erlenmeyer flask experiments. The molasses-based medium was prioritized for bioreactor-scale cultivation due to its significantly higher biomass yield in preliminary trials and its cost-effectiveness, offering a more economically viable alternative for large-scale production.

The YM medium yielded a wet biomass of 14.20 ± 0.01 g and a dry biomass of 4.139 ± 0.001 g per liter of culture. In comparison, the molasses-based medium resulted in a higher wet weight of 15.69 ± 0.01 g and a dry weight of 4.865 ± 0.001 g, indicating an improved growth performance. The enhanced biomass formation in the molasses medium can be attributed to its rich content of fermentable sugars and trace nutrients, which likely supported more robust cell proliferation.

These findings reinforce the potential of molasses as a low-cost, high-yield alternative to conventional synthetic media for industrial-scale cultivation of *Xanthophyllomyces dendrorhous*. Beyond economic benefits, the utilization of molasses aligns with sustainable bioprocessing practices by repurposing agro-industrial byproducts.

3.4. Carotenoid Extraction

Astaxanthin extraction was performed using dimethyl sulfoxide (DMSO), which proved to be highly effective in disrupting the rigid cell wall of *Xanthophyllomyces dendrorhous*. This method facilitated efficient pigment release, resulting in maximal carotenoid recovery. The extracted pigment was analyzed using high-performance liquid chromatography (HPLC), and the chromatogram confirmed the presence of astaxanthin as the dominant carotenoid compound (Figure 3). Retention time and peak area were consistent with standard astaxanthin, validating the identity and purity of the extract.

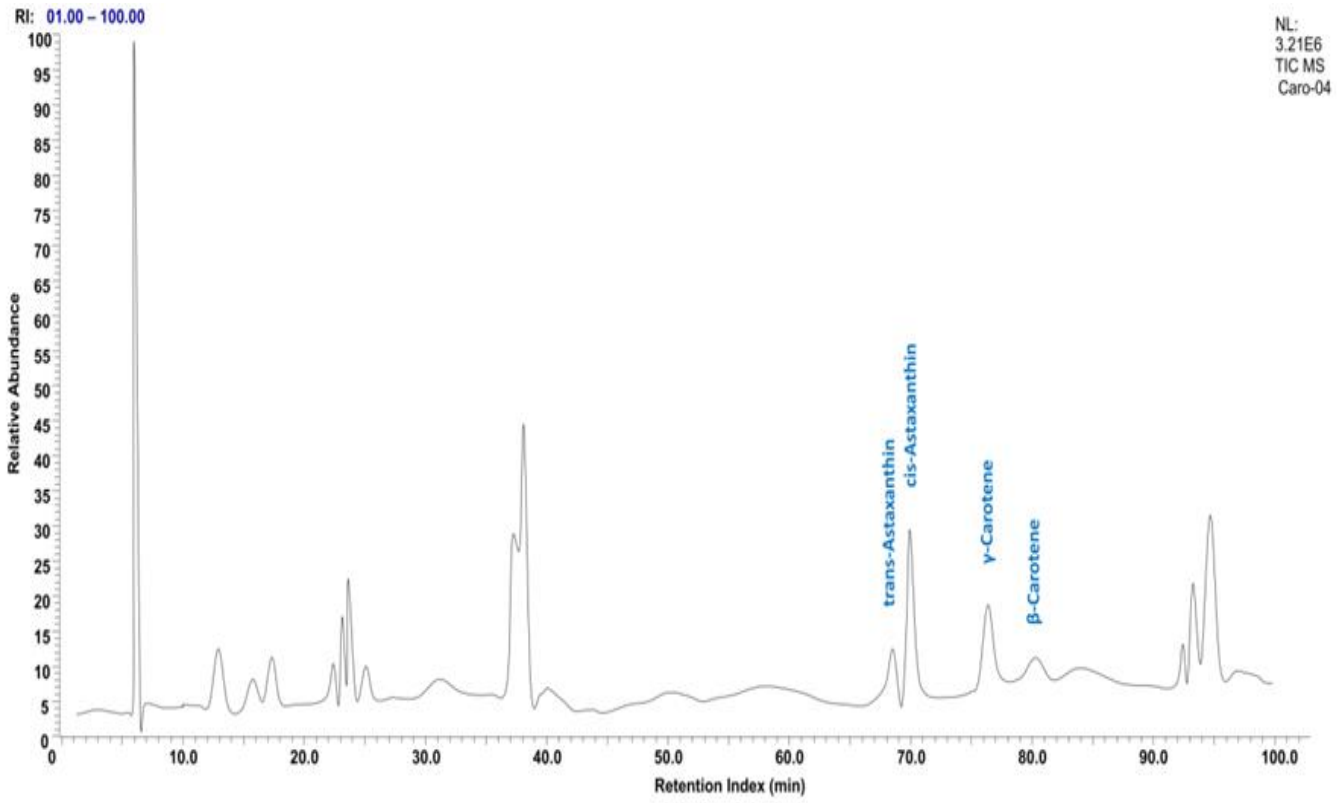

Figure 3. Representative HPLC chromatogram of the carotenoid extract obtained after 120 days of cultivation of *Xanthophyllomyces dendrorhous* in the optimized YM medium at 22 °C. Peaks corresponding to trans-astaxanthin, cis-astaxanthin, β-carotene, and γ-carotene are indicated.

The HPLC chromatogram of the carotenoid extract revealed four distinct peaks corresponding to trans-astaxanthin, cis-astaxanthin, β-carotene, and γ-carotene. Each peak was further identified and confirmed by mass spectrometry (MS) analysis, providing molecular-level validation of the extracted compounds. The trans-astaxanthin peak showed a major molecular ion at m/z 596.9, consistent with its molecular formula ($C_{40}H_{52}O_4$). The cis-isomer of astaxanthin exhibited the same molecular ion peak, but with a slightly different fragmentation profile, allowing for isomer distinction. The β-carotene and γ-carotene peaks both displayed a molecular ion at m/z 536.9, yet their distinct MS fragmentation patterns enabled their individual identification. These results validate the efficiency of the extraction protocol and confirm the chemical identity of the bioactive pigments present in the sample.

3.5. Antioxidant Activity

To assess the bioactivity of the extracted pigment, DPPH radical scavenging activity was measured. The astaxanthin extract exhibited a remarkable antioxidant activity of 96%, indicating its high efficiency in neutralizing free radicals. This strong performance underscores its potential as a natural antioxidant in various industries.

To contextualize its potency, the antioxidant activity of astaxanthin was compared to that of other well-known antioxidant compounds (Table 6). The results clearly demonstrate the superior radical-scavenging capacity of astaxanthin, exceeding that of vitamins C and E as well as β-carotene.

Table 6. Comparative antioxidant activity of astaxanthin and selected antioxidants (DPPH assay)

Compound	DPPH Scavenging Activity (%)	Reference Concentration (µg/mL)
Astaxanthin (extracted)	96%	50
Vitamin C	85%	50
Vitamin E (α-Tocopherol)	78%	50
β-Carotene	68%	50

These findings support the feasibility of using cost-effective culture conditions (molasses-based media) to produce a high-value bioactive compound with superior antioxidant functionality. This not only enhances production scalability but also provides a sustainable source for pharmaceutical and nutraceutical applications.

4. Conclusion

In this study, we successfully optimized the culture conditions for astaxanthin production by *Xanthophyllomyces dendrorhous* through a combination of statistical modeling and the use of cost-effective carbon sources. Response RSM identified the optimal levels of glucose, yeast extract, and pH that significantly enhanced biomass and pigment yield. Furthermore, the replacement of refined glucose with sugarcane molasses and other inexpensive substrates proved effective without compromising pigment output. Among the evaluated extraction methods, DMSO-based extraction provided the highest carotenoid recovery. Validation in a stirred-tank bioreactor confirmed the applicability of the optimized conditions at a larger scale. This study just focused on application of single strain *Xanthophyllomyces dendrorhous*, while coculture may show new windows in effective production. Overall, this approach provides a cost-efficient and scalable strategy for microbial astaxanthin production, supporting its industrial application in functional foods, supplements, and cosmetic formulations. Future research could be oriented to testing other strains or extraction methods.

5. Declarations

5.1. Acknowledgments

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5.2. Authors' Contributions

All authors equally contributed to this work.

5.3. Declaration of Interest

The authors of this article declared no conflict of interest.

5.4. Ethical Considerations

All ethical principles were adhered in conducting and writing this article.

5.5. Transparency of Data

In accordance with the principles of transparency and open research, we declare that all data and materials used in this study are available upon request.

5.6. Funding

This research was carried out independently with personal funding and without the financial support of any governmental or private institution or organization.

5.7. Using Artificial Intelligent chatbots

No AI chatbot has been used in this study.

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