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Optimization of Carbon and Nitrogen Sources, Yeast Extract Concentration, and Agitation Speed for Carotenoid Production by Rhodotorula mucilaginosa

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ABSTRACT: This study aimed to evaluate the effects of carbon sources, yeast extract concentration, nitrogen sources, and agitation speed on carotenoid biosynthesis by yeast Rhodotorula mucilaginosa, due to the high cost and limited availability of plant-based sources that produce carotenoids. The results demonstrated that sucrose was the most effective carbon source for carotenoid production, yielding 1250.95 µg/L, followed closely by date molasses, which produced 1207.91 µg/L. In contrast, lactose was the least effective carbon source, resulting in the lowest carotenoid yield (98.83 µg/L). The optimum yeast extract concentration for carotenoid production was 5 (g/L), while ammonium sulfate proved to be the most suitable nitrogen source, achieving the highest carotenoid production of 1505.01 µg/L. Furthermore, the optimum agitation speed was 250 rpm. Overall, these findings indicate that maximizing carotenoid production by R. mucilaginosa depends on the appropriate selection of carbon and nitrogen sources, optimal yeast extract supplementation, and suitable aeration conditions achieved through proper agitation, thereby providing favorable nutritional and environmental conditions for microbial growth and carotenoid biosynthesis.

INTRODUCTION

Dyes are compounds widely used in many industries, including textiles, food processing, and pharmaceuticals. The global market for dyes reached 1.5–2 billion kg in 2019–2024, representing an annual growth rate of 4.2% (25). Carotenoids are among the most prominent of these dyes due to their wide spectrum of colors, ranging from yellow to orange to red (33).

Carotenes are organic compounds that are insoluble in water and soluble in organic solvents. They are widely found in many plants and are converted into vitamin A in the liver. They consist of unsaturated hydrocarbon chains, and the hydrogen atoms vary according to the number of double bonds. They have different shapes and are responsible for the color gradient, ranging

from yellow to red. The general chemical formula for carotenes is C40Hx tetraterpenes, and each terpene consists of two isoprene units (18). The most important sources of carotenoids are synthetic and natural. Synthetic carotenoids are chemical substances with a high pigmentation capacity, but they are expensive and considered carcinogenic and harmful to the human body (32). Natural carotenoids, on the other hand, are of plant origin, such as those extracted from fruits and vegetables like bell peppers (red or orange), carrots, and melons. These are economically expensive due to the high cost of raw materials, particularly the growing season and extraction costs. These factors have prompted researchers to seek alternative sources for these pigments (21). Or microbial-sourced carotenoids, which have been considered a less expensive alternative to plant-sourced carotenoids, especially in terms of pigment productivity, with lower raw material costs and cheaper growing media, often relying on byproducts and industrial and agricultural waste, in addition to date molasses (31). These sources also contribute to boosting the immune system thanks to their antioxidant properties and their ability to bind to free radicals, thus enhancing their ability to reduce the risk of diseases, including cancer. Furthermore, these sources extend the shelf life of food by inhibiting the growth of pathogenic microbes that contaminate it. They are also characterized by their strong and stable color, especially when used in juices as a natural coloring agent (2) ; (3).

Many microorganisms, particularly certain types of yeast known as Basidiomycota, which are classified as infrared yeasts, can produce accumulated carotenoids (13) ; (28). The genera Rhodosporidium and Rhodotorula sp. are among the most studied carotenoid-producing strains, resulting in orange-colored colonies (12) ; (30). This study aimed to produce microbial carotenoids with high staining power comparable to synthetic dyes.

Although numerous studies have optimized carotenoid production in *Rhodotorula spp.*, limited information is available regarding the comparative use of low-cost substrates such as date molasses together with optimization of nitrogen source, yeast extract concentration and agitation speed under identical fermentation conditions, therefore the study aimed to identify the factors affecting the biological production of carotenoids, given their biological importance and multiple uses as alternative sources to plant carotenoids, which are expensive and costly, and are comparable to synthetic dyes in terms of staining power.

MATERIALS AND METHODS

Solutions and Chemical Reagents Used

0.1N Sodium Hydroxide (NaOH) Solution

The solution was prepared by adding 4 grams of NaOH to 250 ml of distilled water until completely dissolved in a 1000 ml volumetric flask. The flask was then filled with distilled water up to the mark on the volumetric flask. This solution was used to adjust the pH of culture and growth media (22).

0.1N Hydrochloric acid (HCl) solution

The solution was prepared by adding 8.8 ml of concentrated HCl to 250 ml of distilled water in a 1000 ml volumetric flask and then topping it up with distilled water to the mark. This solution was also used to adjust the pH of culture media (22).

Stock glucose solution

Prepared by dissolving 0.064 grams of glucose in a specific quantity of distilled water until completely dissolved in a 100 ml volumetric flask, then completing the volume with distilled water to the mark (35).

Normal saline solution

The solution was prepared by dissolving 0.85 g of sodium chloride (NaCl) in a quantity of distilled water in a 100 ml volumetric flask. The volume was then topped up with distilled water to the mark and sterilized by autoclave at a temperature of 121°C, a pressure of 15 psi, and a time of 15 minutes (35).

Sterilization Methods Used

Wet Sterilization by Autoclave

Cultural media, solutions, reagents, and glassware were sterilized using an autoclave at a pressure of 15 psi and a temperature of 121°C for 15 minutes (35).

Chemical Sterilization

70% ethyl alcohol was used to sterilize the incubator, hood, and instruments before use.

Preparation of Culture Media:

The culture media used in this study were prepared according to the instructions in (35), except for those media for which specific preparation procedures were indicated.

Yeast-Malt Extract Broth (YMB) Medium

This medium was prepared by dissolving 3 grams of yeast extract and adding 3 grams of malt extract, 10 grams of glucose, and 5 grams of peptone in 1000 ml of distilled water in a volumetric flask. The pH was adjusted to 6. This medium was prepared for the preservation of isolates (27).

Yeast-Malt Extract Ager (YMA) Medium

This medium was prepared with the same ingredients and quantities approved for the (YMB) medium in the previous step, and 20 grams of agar were added to each 1000 ml of culture medium (27).

Minimal media (Davis formulation)

Prepared by dissolving 0.1 g of dextrose, 0.1 g of ammonium sulfate, 0.7 g of dipotassium phosphate, 0.2 g of monopotassium phosphate, 0.05 g of sodium citrate and 0.01 g of magnesium sulfate, all in 100 ml of distilled water, and adjusting the pH to 7. This medium was used to activate the vaccine before the vaccination process, according to the instructions of the German company Merck (10).

Yeast Sources

The yeast was previously isolated from its sources, purified, and characterized according to (11).

Starter culture preparation

The isolate was prepared at all stages of screening in subsequent experiments according to what was mentioned by (24).

Inoculum preparation for the experiments

The purified colony, grown on a slanted YMA medium, was transferred ten times by bacterial carrier and incubated at 30°C for 48 hours into a 250 ml conical flask containing 50 ml of YMB. These flasks were incubated in a shaking incubator at 30°C and a mixing speed of 100 rpm for 24 hours, and the absorbance was estimated at a wavelength of 570 nm (11).

Cell Dry Weight Estimation

The cell dry weight was estimated in 50 ml of fermentation culture after centrifugation at 3500 rpm for 15 minutes. The sediment was washed several times with distilled water, and the filtrate was discarded each time after centrifugation. The sediment was then dried at 85°C until a steady weight was reached. The cell dry weight was estimated based on g/L of culture volume (6).

Estimation of Carotenes

The amount of carotenes was estimated according to (31), based on the standard beta-carotene curve prepared with graded concentrations including 10, 8, 6, 4, 2 micrograms/ml, and the amount of carotene was calculated using the standard curve. The standard curve was prepared by taking 0.1 grams of standard beta-carotene and placing it in a volumetric flask. This amount was dissolved using the organic solvent hexane. A small amount of hexane was taken until it dissolved using a mixer, and then the solution was added to the mark in a 100 ml flask. This solution was called Solution A. Then another 100 ml flask was prepared, and 1 ml of Solution A was taken and placed in the second flask, which was called Solution B. Each 1 ml of Solution B contains 10 micrograms of standard beta-carotene. After completing Solutions A and B, a series of dilutions and concentrations were carried out with (2, 4, 6, 8) micrograms/ml of the organic solvent hexane, as follows:

2µg/ml (1 ml of solution B + 4 ml hexane).

4µg/ml (2 ml of solution B + 3 ml hexane).

6µg/ml (3 ml of solution B + 2 ml hexane).

8µg/ml (4 ml of solution B + 1 ml hexane).

10 µg/ml (solution B).

Then the absorbance was read at a wavelength of 450 nm and the curve was plotted as in Figure (1). The following law was then applied to calculate the total amount of carotene:

$$\mathbf{M = RFv_s * 1000 \div V_c}$$

Where:

M. Total carotene quantity (µg/L).

R. Spectrophotometric reading (nm).

F. Factor, value 4.80 µg carotene/ml solvent.

V_s. Total solvent volume (ml).

V_c. Culture volume (ml).

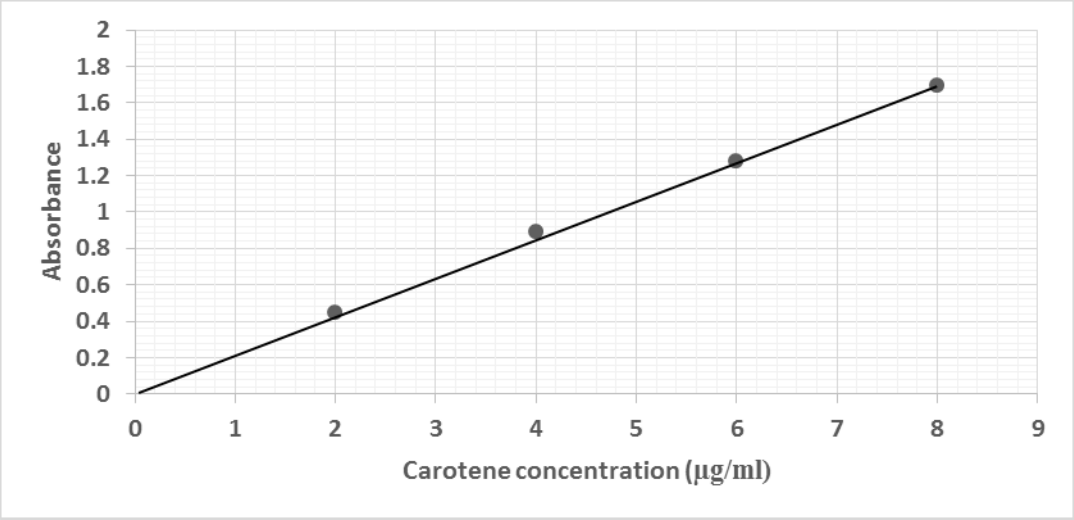

Figure (1) Standard curve for beta-carotene estimation

Estimation of Residual Carbohydrates

The residual carbohydrates after fermentation were estimated using the phenol-concentrated sulfuric acid method (7). One milliliter of the fermentation solution was taken after cell removal by centrifugation. Dilutions were made using distilled water. One milliliter of the final dilution was then taken, and one milliliter of a 5% phenol solution was added and thoroughly mixed. Five milliliters of concentrated sulfuric acid were then added, and the tubes were vigorously shaken. They were placed in a water bath at 25-30°C for 30 minutes. The optical density was measured at a wavelength of 490 nm using a UV/vis spectrophotometer. The amount of carbohydrates in the fermentation samples was then calculated using a standard curve with pure glucose as the standard sugar. The standard curve was prepared using different concentrations of the storage sugar solution (32-160 µg/ml), and this method was applied to obtain the density values, all experiments were performed in triplicate. The photochemicality of these different concentrations of glucose is shown in Figure (2).

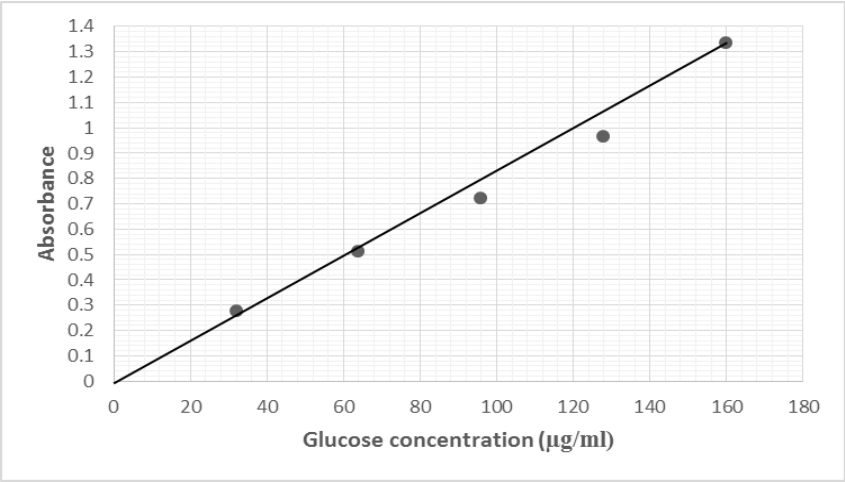

Figure (2) Standard curve for estimating total carbohydrates

Basic fermentation medium for carotenoid production

The medium was composed of 25 g glucose, 10 g yeast extract, 2 g monohydrogen potassium phosphate, 2 g dihydrogen potassium phosphate, and 0.1 g hydrated magnesium sulfate, all dissolved in 1 liter of distilled water. The growth conditions were adjusted as follows: inoculum volume 10%, temperature 30°C, pH 6, incubation period 4 days, and a shaker incubator speed of 150 rpm (5).

Effect of yeast extract concentration

Several different concentrations of yeast extract were studied, and the concentrations were as follows: (2.5, 5, 7.5, 10, 12.5) g/L of medium volume. These concentrations were monitored in the fermentation medium, and the optimal concentration was determined and replaced with other nitrogen sources.

Effect of Nitrogen Sources

To determine the optimal nitrogen source for the biosynthesis of carotenoids that yields the highest productivity, different nitrogen sources were added, each source added to the medium separately. The nitrogen sources that replaced yeast extract were: ammonium chloride, ammonium sulfate, urea, meat extract, malt extract, and casein hydrolysate. The optimal source was used in subsequent tests.

Effect of Rotation Speed of the Shaking Incubator (Aeration)

The optimal rotation speed of the shaking incubator for carotenoid production was determined by testing several speeds: (50, 100, 150, 200, 250) rpm. The optimal speed was then used in subsequent tests for the production process.

RESULTS AND DISCUSSION

Effect of Carbon Sources

Table (1) shows that the best carbon source for the bioproduction of carotenoids is sucrose, with a carotenoid content of 1250.95 µg/L. This contrasts with the other carbon sources used in the study, and this result agrees with (9), who indicated that sucrose outperformed other sugars in carotenoid production compared to glucose, which they considered a control treatment. This was confirmed by (20), who indicated that sucrose and glucose are among the most effective carbon sources for high carotenoid production by Rhodotorulla yeast.

While the results obtained by (8) differed, It was shown that sucrose gave less carotenoids compared to the use of monosaccharides of sucrose and fructose when growing Rhodotorulla yeast on media containing these sugars individually and under the same conditions adopted for growth.

The same table showed that the highest ratio of carotenoids to sugar consumed was for sucrose, compared to other carbon sources, reaching 51.05 micrograms/g. The lowest ratio was for lactose, at 9.88 micrograms/g. Lactose also yielded the lowest values for biomass, carotenoids, and sugar consumed, along with the highest pH value, this is attributed to the high capacity of Rhodotorola mucilaginosa yeast and its unique metabolic properties for hydrolyzing sucrose by the enzyme invertase into glucose and fructose. These are rapidly transported into yeast cells and enter the glycolysis pathway. This increases the efficient uptake of these monosaccharides and the production of adenosine triphosphate (ATP), pyruvate, and acetyl coenzyme-a, the two main components of the mevalonate (MVA) pathway responsible for carotenoid biosynthesis. This leads to greater carbon consumption, increased biomass formation, and carotenoid accumulation, resulting in higher carotenoid production relative to the amount of sugar consumed. (15) ; (37). Recent studies have indicated that sucrose and glucose are the most commonly used and effective sources of carbon for carotenoids by Rhodotorola, due to their high absorption efficiency and ability to stimulate pigment accumulation (4), In contrast, Rhodotorola mucilaginosa has a limited capacity to metabolize lactose efficiently, resulting in slower lactose uptake, reduced glucose availability, decreased biomass formation, and consequently, reduced carotenoid production. (15). This is attributed to the fact that the yeast Rhodotorulla mucilaginosa lacks the enzyme β-galactosidase, which breaks down lactose into its monosaccharides, glucose and galactose. This result aligns with the findings of (9), who confirmed that lactose-digesting yeasts are rare under optimal growth conditions. (14) indicated that *Rhodotorulla lactis* is the only species capable of metabolizing lactose. Maltose sugar came in third place with the lowest percentage of biomass and carotenoid production, reaching 8.99 and 899.95 respectively. This is attributed to the fact that it is a disaccharide, and when it is metabolized and digested, it is converted into two glucose molecules. Thus, the sugar content increases by double the percentage calculated based on the medium. Consequently, the increased sugar concentration leads to an increase in osmotic pressure, which in turn leads to weak yeast growth and weak production of carotenoids.

The same table shows that the biomass of date molasses surpassed other carbon sources, reaching 13.05 g/L. This is attributed to its high content of carbon compounds, including sucrose, nitrogenous compounds, minerals, and vitamins. These components supported the yeast in maintaining its logarithmic phase and continuous division, at the expense of carotenoid production, which occurs during the resting phase and cessation of division. Carotenoids are essential for pigment production. This finding aligns with that of (19), who indicated that using date molasses reduces the need for additional carbon sources while providing high biomass and a suitable growth medium.

Table (1) Effect of carbon sources on the bioproduction of carotenoids using the basic medium as a fermentation medium. Temp.=30°C, pH=6, rpm=150

Carbon source	Biomass (g/L)	Carotenoids (µg/L)	Consumed Sugar (g/L)	Carotenoids/Consumed Sugar (µg/g)	Final pH
Sucrose	12.80 a	1250.95 a	24.50 a	51.05 a	5.93 bc
Maltose	8.99 b	899.95 c	21.91 b	41.09 b	5.90 bc
Galactose	12.99 a	1149.05 b	24.32 a	47.28 ab	5.89 bc
Lactose	3.65 d	98.83 e	10.01 c	9.88 d	7.50 a
Cane molasses	13.04 a	1200.51 a	24.31 a	49.38 a	5.80 c
Beetroot molasses	4.90 c	250.50 d	20.90 b	11.98 d	6.03 b
Sweet syrup	13.05 a	1207.91 a	24.52 a	49.30 a	5.50 d

***Different lowercase letters within the same column indicate significant differences (p≤ 0.05) between the effects of the treatments**

The lowest pH value (5.50) was observed in the date molasses medium, which may be attributed to the active assimilation of sugars and nutrients by Rhodotorula mucilaginosa. During growth, yeast metabolism can alter the culture pH through the uptake of nutrients and the production of organic metabolites and ionic compounds, creating conditions that favor cellular growth. The superior performance of date molasses compared with the other tested substrates is likely related to its high content of readily metabolizable monosaccharides, particularly glucose and fructose, which are rapidly utilized as carbon and energy sources.

Furthermore, the highest sugar consumption among the tested agro-industrial substrates was recorded in the date molasses medium (24.52 g/L), second only to sucrose and exceeding both beetroot and sugarcane molasses. These findings indicate that date molasses is a promising, economical, and sustainable substrate for the cultivation of R. mucilaginosa. Although studies evaluating date molasses specifically for R. mucilaginosa are currently lacking, recent investigations have demonstrated that molasses-based media are effective low-cost substrates for the cultivation of R. mucilaginosa, supporting high biomass production and the synthesis of valuable metabolites (16) ; (40). Therefore, the present findings extend the potential application of molasses-based media by demonstrating that date molasses can serve as an efficient alternative carbon source for the growth of R. mucilaginosa.

Effect of Yeast Extract Concentration

Table (2) below shows different concentrations of yeast extract, including 2.5, 5, 7.5, 10, and 12.5 g/L. Concentration 5 yielded the highest carotenoid content at 1600.01 µg/L. The carotenoid content decreased with increasing yeast extract concentration, reaching its lowest level at the highest concentration of 1199.02 µg/L. The same table also shows that the highest carotenoid-to-sugar ratio was observed in the medium containing 5 g/L of yeast extract, reaching 64.49 µg/g. This result is consistent with the findings of (1), who indicated that both high concentrations (above 6 g/L) and low concentrations negatively impact total carotenoid production by Rhodotorulla yeast.

It is observed from the table that the high percentages of yeast extract are directly proportional to the increase in the percentages of yeast biomass, as the concentrations of 10 and 12.5 g/L each obtained a biomass of 12.91 and 13.00 g/L respectively. This is due to the abundance of nutrients in the growth medium of yeast extract, such as vitamins, amino acids, and other factors that help to keep the microorganism in continuous division in the logarithmic phase and building cells without going through the stationary phase, which directs the microorganism to produce secondary metabolic products and produce carotenoids. As for the concentration of 2.5 g/L, it gave the lowest percentage of biomass, as it gave 10.30 g/L. This is attributed to the lack of sufficient nutrients and nitrogenous materials for yeast growth, which leads to a deficiency in yeast biomass, These findings are consistent with recent studies demonstrating that sufficient yeast extract supplementation enhances biomass production in Rhodotorula mucilaginosa and other oleaginous red yeasts by providing essential nutrients required for cellular growth and metabolism (15) ; (40).

Table (2) Effect of yeast extract on the bioproduction of carotenoids using basic culture medium as a fermentation medium. Temp.=30°C, pH=6, rpm=150

Effect of yeast extract (g/L)	Biomass (g/L)	Carotenoids (µg/L)	Consumed Sugar (g/L)	Carotenoids/Consumed Sugar (µg/g)	Final pH
2.5	10.30 c	1270.91 c	23.93 b	53.10 c	5.01 d
5	12.33 b	1600.01 a	24.81 a	64.49 a	5.50 c
7.5	12.64 ab	1435.09 b	24.63 a	58.26 b	5.90 b
10	12.91	1301.81	24.42	53.30	5.92

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	a	bc	a	c	b
12.5	13.00 a	1199.02 c	24.15 ab	49.64 d	6.21 a

***Different lowercase letters within the same column indicate significant differences (p≤ 0.05) between the effects of the treatments**

(39) indicated that increased nitrogenous compounds lead to increased biomass compared to low levels of carotenoids due to the gradual depletion of nitrogen sources.

(17) reported that increasing the yeast extract concentration from 5–10 g/L resulted in an increase in total carotenoids from 702–732 µg/L. The carotenoids increased slightly when the yeast extract concentration was increased to 15 g/L, reaching 745 µg/L when Rhodotorulla mucilagenosa was cultured in a medium containing 20 g of glucose.

The pH decreased at all concentrations of yeast extract used except for the highest concentration of 12.5 g/L, which showed a significant increase to 6.2. This is attributed to the accumulation of ammonia resulting from the yeast's biological activities and the high concentration of amino acids in the yeast extract, which leads to increased alkalinity in the growth medium. Therefore, we conclude from these results that the optimal concentration of yeast extract is 5 g/L, which yielded the highest production of carotenoids. We will use this same concentration to replace nitrogen compounds in subsequent experiments.

Effect of Nitrogen Sources

Nitrogen is an essential nutrient for microbial growth, as it plays a fundamental role in cell synthesis, maintenance, and division. Biomass production cannot be sustained without an adequate nitrogen source because nitrogen is required for the biosynthesis of amino acids, which are the building blocks of proteins, as well as nitrogenous bases that constitute nucleic acids (DNA and RNA). In addition, nitrogen is involved in the synthesis of several vitamins, coenzymes, and other cellular constituents necessary for metabolism and cell proliferation. Therefore, the availability of an assimilable nitrogen source in the culture medium is a key factor governing microbial growth and biomass accumulation (34). This experiment investigated the effects of various organic and inorganic nitrogen sources. The most suitable source for carotenoid production was identified from a locally isolated yeast isolate, *Rhodotorulla mucilaginosa*. The study included several nitrogen sources, namely ammonium sulfate, urea, ammonium chloride, malt extract, meat extract, and casein hydrolysate.

Table (3) below shows that the nitrogen source with the highest carotenoid yield was ammonium sulfate, with a carotenoid content of 1505.01 µg/L and a ratio of 64.04 µg/g to consumed sugar, respectively. Urea followed in yield, with a carotenoid content of 1204.71 µg/L. These results are consistent with those of (9), who obtained the highest carotenoid yield when growing Rhodotorulla mucilaginosa yeast on a medium containing ammonium sulfate, with urea being the second most productive medium. All these results are consistent with those of the current study.

Table (3) Effect of nitrogen source on carotenoid biosynthesis using basic medium as a fermentation medium Temp.=30°C, pH=6, rpm=150

Nitrogen source	Biomass (g/L)	Carotenoids (µg/L)	Consumed Sugar (g/L)	Carotenoids/Consumed Sugar (µg/g)	Final pH
Ammonium sulfate	7.95 b	1505.01 a	23.50 a	64.04 a	3.51 d
Urea	8.04 b	1204.71 b	24.30 a	49.57 b	6.01 b
Ammonium chloride	5.90 c	701.01 d	23.04 ab	30.42 d	3.70 d
Malt extract	3.10 d	79.81 e	20.01 b	3.98 e	6.50 a

Meat extract	9.95 a	901.01 c	24.03 a	37.49 c	6.10 b
Casein hydrolysate	8.94 ab	501.01 d	24.01 a	20.86 d	5.57 c

***Different lowercase letters within the same column indicate significant differences (p≤ 0.05) between the effects of the treatments**

(16) also noted that adding 3% ammonium sulfate increases the percentage of carotenoids from 81.16% to 94.14% in the yeast *Rhodotorulla mucilaginosa*, where ammonium sulfate is considered a yeast growth stimulant.

Table (3) shows that the malt extract gave the lowest percentage of carotenoids, which reached 79.81 micrograms/liter, and also gave the lowest percentage of biomass, which reached 3.10 g/liter. The reason for this is that the yeast was unable to metabolize maltose sugar well, which led to a decrease in all the mentioned percentages. As for the meat extract, it gave the highest percentage of biomass, which reached 9.95 g/liter, and it surpassed all the other treatments in terms of growth. This is attributed to the meat containing amino acids important for growth along with other growth factors, which led to supporting cell growth and building and producing proteins, as biomass is greatly affected by the concentration and type of carbon source (36).

The results showed that the pH of the ammonium sulfate treatment was the least acidic, at 3.51. This is attributed to the release of sulfate radicals, which then combine with nitrates after the yeast consumes ammonium as a nitrogen source. Yeast requires ammonium for biosynthesis and metabolism, and these two factors contribute to a lower pH compared to other treatments in the medium. The highest pH value was observed in the presence of malt extract. This increase is due to the decomposition of nitrogenous compounds, leading to ammonia accumulation in the production medium (23).

Effect of Aeration Rate

Microorganisms require oxygen during their growth and the production of important metabolic products. Aeration in the fermentation medium is crucial for optimal carotenoid production by the yeast *Rhodotorulla mucilaginosa*, which is an aerobic microorganism and requires oxygen during its growth and carotenoid biosynthesis (38). This study investigated the effect of five different rotation speeds of a shaker incubator on carotenoid growth and biosynthesis.

Table (4) shows that the optimal yield of carotenoids, biomass, and production efficiency was achieved at a shaker incubator speed of 250 rpm, reaching 1999.90 µg/L, 8.50 g/L, and 81.62 µg/g, respectively. Conversely, using a speed of 50 rpm resulted in a decrease in all values, with carotenoid yield, biomass, and production efficiency dropping to 1000.05 µg/L, 5.50 g/L, and 44.84 µg/g, respectively. This decrease is attributed to the reduced oxygen levels in the fermentation medium. The shaker incubator's rotation speed not only supplies the fermentation medium with oxygen but also mixes the components, providing nutrients in direct contact with the yeast cells to enable them to perform their biological and metabolic activities at a high level. This study showed that increasing the ventilation rate increases the rate of production of carotenoids produced by the yeast *R. mucilaginosa.*

Table (4) Effect of aeration on the bioproduction of carotenoids using the basic medium as a fermentation medium
Temp.=30°C, pH=6, rpm=150

Mixing speed (rpm)	Biomass (g/L)	Carotenoids (µg/L)	Consumed Sugar (g/L)	Carotenoids/Consumed Sugar (µg/g)	Final pH
50	5.50 d	1000.05 e	22.30 c	44.84 e	2.50 a
100	7.07 c	1590.44 d	23.20 bc	68.55 d	2.40 ab
150	7.99 b	1670.90 c	23.50 b	71.10 c	2.35 bc

200	8.20 ab	1799.99 b	24.20 a	74.37 b	2.31 cd
250	8.50 a	1999.90 a	24.50 a	81.62 a	2.25 d

***Different lowercase letters within the same column indicate significant differences (p≤ 0.05) between the effects of the treatments**

This study agrees with the findings of (29), who indicated that carotenoid production rates increase with increasing mixing speed. They demonstrated that the highest carotenoid yields were achieved at very high stirring speeds, reaching up to 700 rpm. This was further corroborated by (26), who noted that high aeration rates increase the specific growth rate of carotenoids and biomass when producing carotenoids from the yeast *Rhodotorulla mucilaginosa* MTCC.

CONCLUSION

This study concluded that the best carbon source for the bioproduction of carotenoids is sucrose and date molasses, and the best concentration of yeast extract was 5 (g/L), and the best nitrogen source was ammonium sulfate, and the best mixing speed was (250 rpm), Therefore, this findings is important as alternative for caretoniod production from natural sources. Add future study or next analysis: for example: Future studies should investigate response surface methodology, bioreactor scale-up, metabolomic analysis, and economic feasibility of carotenoid production using date molasses.

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