

## Effect of Extraction Conditions on Carotenoids from *Rhodotorula Mucilaginosa*

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### ABSTRACT

Carotenoids are a group of 40-carbon isoprenoids with high lipid solubility, widely found in fruits, vegetables, etc. They have an unsaturated structure with strong antioxidant activity that helps prevent low-density lipoprotein oxidation and protect cells from free radicals. Currently, carotenoids are not only synthesized from natural sources such as plants but also from microorganisms such as bacteria, yeast, algae, etc. One more attention, becoming an important research area, using microorganisms to produce carotenoids has advantages over than plants because it saves costs and can be more easily expanded to an industrial scale. Therefore, in this study, the influence of extraction conditions such as organic acid, ultrasound time, and solvent on the carotenoid extraction from the yeast *Rhodotorula mucilaginosa* was investigated. The result showed that the process of carotenoid extraction follows 3M citric acid combined with 30 minutes of ultrasound for cell disruption; complete extraction process in 100% ethanol with dry cell weight/ethanol ratio of 1/50 g/mL. The total carotenoid content reached  $548.98 \pm 6.30 \mu\text{g/g}$ .

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## 1. Introduction

*Rhodotorula mucilaginosa* is capable of biosynthesizing specific carotenoids, such as  $\beta$ -carotene, torulene and torularhodin in various ratios and producing  $\beta$ -carotene using large fractions [1]. Carotenoids are a family of natural organic pigments that play an important role in life and are found in plants, algae, rubies, persimmon, bacteria and some photosynthetic organisms. Carotenoids are considered color nutrients because they have many similar properties to vitamins that create yellow, orange, and red colors in both the plant and animal kingdoms. In plants, carotenoids are found in leaves along with chlorophyll, in flowers, fruits and vegetables. In the animal body, carotenoids are dissolved in fat or protein compounds in the aqueous phase. Humans cannot naturally synthesize carotenoids and depend on dietary sources of nutrients. There are currently about 850 naturally occurring carotenoids reported up to 2018 and they are divided into two main groups: xanthophyll and carotene [2].

Compound carotenoids can continue to be found and obtained from a variety of sources. We can get it from chemical synthetic sources (accounting for 70 ÷ 80%) and natural sources (using only 20 ÷ 30%). In plants, carotenoids are abundant in fruits and vegetables. Typically, in carrots, the  $\beta$ -carotene content reaches 87  $\mu\text{g/g}$ ; Gac fruit contains  $\beta$ -carotene content of about  $101 \pm 38 \mu\text{g/g}$  and lycopene content of about  $380 \pm 71 \mu\text{g/g}$ ; in tomatoes, the  $\beta$ -carotene content is 62  $\mu\text{g/g}$  and the lycopene content reaches 114.4  $\mu\text{g/g}$  [3], [4]. In animals, carotenoids contribute to the brilliant colors observed in the fur, skin, white or feathers of many animals such as birds or fish,... The carotene group is abundant in fish liver, especially mackerel liver and in the liver of animals such as chickens, ducks, pigs and in some other species such as fish, eggs, insects, poultry, etc [5]. The carotenoid production process of microorganisms takes place in cells, so to recover carotenoids from microorganisms, cells need to be broken to create conditions for the solvent to penetrate and dissolve carotenoids. Most traditional extraction methods have used processes based on volatile organic solvents as solubilizers. Besides, in recent years, many researchers have searched for new and effective replacement techniques, especially: (a) replacing volatile organic solvents with greener, bioavailable solvents. compatible and less toxic waste, such as supercritical waste, biological media or ionic waste and (b) reduce the amount of solvent media required

through the combination process of chemical extraction with new physical processes (such as extraction with the sophistication of microwaves and ultrasound) or biological straining processes (such as extraction with enzyme activity) [6].

Carotenoids are tetraterpene compounds ( $C_{40}H_{56}$ ) formed by the combination of eight isoprene units [7]. Carotenoids have a straight chain structure, eight 5-carbon isoprenoid units linked together, and through the center, there is an alternation of double and single bonds. Some carotenoids in structure contain six-ring edges or contain more oxygen such as alcohol, aldehyde, ketone, carboxylic, and epoxy radicals. From this basic structure, most other carotenoids can be generated through hydrogenation, cyclization, oxidation, or any logical outcome of these processes [5], [8].

The process of extracting carotenoids is often performed using a chromatography column [9]–[14]. Because carotenoid is nonpolar, it is cleaved using polar media such as hexane, acetone, and petroleum ether and measures optimal density (OD) at 400–600 nm [15]. For algae, carotenoid extraction could be performed by using a large amount of cooking oil and performing high-pressure homogenization, which enhances the efficiency of carotenoid extraction from algae. Other extraction methods have also been used such as supercritical CO<sub>2</sub> extraction, pressurized liquids extraction and different solvent extraction [16]–[18]

In this study, we investigated some conditions affecting the extraction of total carotenoids from *R. mucilaginosa* using environmentally friendly chemicals: cell disruption conditions with organic acids combined with ultrasound-assistant method, using ethanol and petroleum ether solvents to replace acetone in the process of extracting total carotenoids to obtain a benign product, easy to apply in food.

## 2. Materials and Methods

### 2.1. Strains and culture conditions

The *Rhodotorula mucilaginosa* ATCC® 66034™ was bought from a Microbiologics (U.S.A). 2% glucose, 1% yeast extract, 1% peptone, and 2% agar were placed on YPD agar plates (Yeast Peptone D-Glucose), *R. mucilaginosa* was cultivated for three days at 30°C before being stored at 4°C. For pre-cultivation, the YPD with 2% D-glucose medium was utilized, and for carotenoid accumulation, the YPD with 10% D-glucose was used. After being pre-cultivated for 24 hours in YPD 2% D-glucose medium with an initial optical density of 660 nm = 0.1, they were cultured in YPD 10% D-glucose medium for carotenoid production as reported of Naoto Urano with some modified [19].

### 2.2. Cultivation and dry cell

Cells from 5 days of cultivation were washed by distilled water twice time and centrifugation at 5000 rpm in 15 min. The dry cell was obtained by Freezing dry by a DC401Freeze Dryer (Yamto, Japan).

### 2.3. Cell disruption

The experiments concerning acid and ultrasound-assisted cell disruption were performed following the procedures described by Hui NI [20] with modifications using an ultrasonic-assisted following Letícia Urnau [21]. 1.5 mL of acid 2.5 M was added into a 15 mL falcon containing 0.1 gram dry cell. Vortex at 1000 rpm for 5 minutes, then let the ultrasound in the Elmasonic S100H (Germany) for 30 min at 30 °C. Then, centrifugating at 3500 rpm for 20 minutes and remove the acid.

### 2.4. Extraction of carotenoids from *R. mucilaginosa*

After cell disruption, 5 mL of acetone solvent was added to the falcon, shake well with a vortex machine at 1000 rpm for 5 minutes. Finally, centrifuge at 3500 rpm for 20 min to remove cell debris to collect the solvent containing carotenoids.[20]

For investigating of cell disruption condition and solvent extracted condition, type of acid (non-acid, citric, lactic, acetic), concentration of acid (1.5; 2.0; 2.5; 3.0; 3.5 M), dry cell weight/acid ratio (1/10;1/15; 1/20 w/v), ultrasound-assisted time (0, 10, 20, 30, 40, 50 min), type of solvent (acetone, ethanol, petroleum, ethanol: petroleum = 1:1) and dry cell weight/solvent (1/40; 1/50; 1//60; 1/70 w/v) were researched.

### 2.5. Determination of total carotenoid content

The absorbances were read on Hitachi UH-5300 UV-VIS spectrophotometer.

Considering solely these major abundant carotenoids and exchanging  $\alpha$ -carotene for a broader carotenoid spectrum, absorption coefficient for  $\beta$ -carotenoid in the acetone solvent  $A_{1cm}^{1\%} = 2500$  (represents the extinction at a certain wavelength and in a certain solvent of concentration 1%, in a cuvette with a diameter of 1 cm, at 454nm) and in the ethanol solvent  $A_{1cm}^{1\%} = 2620$ , in the petroleum ether solvent  $A_{1cm}^{1\%} = 2592$  [8], [15], [22]. The formula for total carotenoid content (TCC) as:

$$TCC (\mu\text{g/g}) = \frac{A * V * 1000 * 1000}{A_{1cm}^{1\%} * P * 100}$$

TCC: total carotenoid content,  $\mu\text{g/g}$

$A_{1cm}^{1\%}$  represents the extinction at a certain wavelength and in a certain solvent of concentration 1%, in a cuvette with a diameter of 1 cm.

A: absorbance of sample at optimum absorption

V: volume of sample, mL

P: weight of dry cell, g

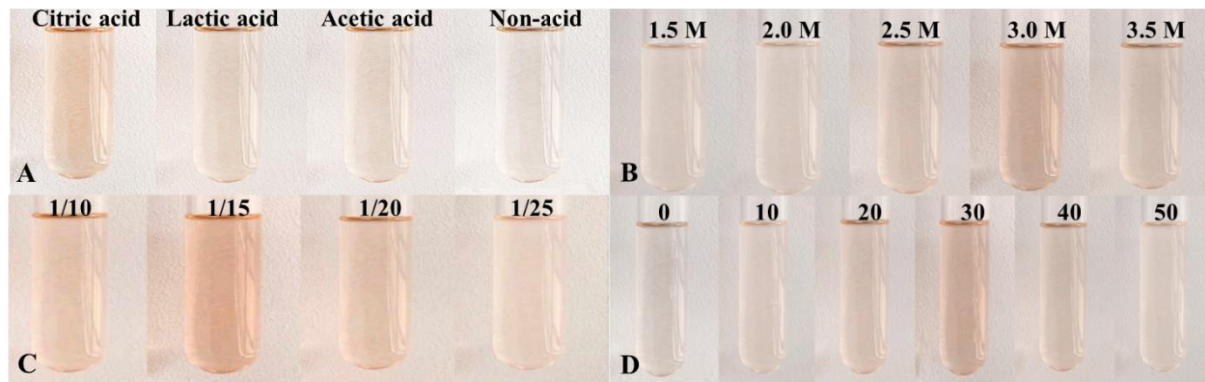
## 2.6. Statistical analysis

All experiments were replicated at least three times. The student's *t*-test was used to determine the statistically significant differences ( $p \leq 0.05$ ).

## 3. Results and Discussion

### 3.1. Effect of organic acids on carotenoid extraction

To investigate the effects of organic acid on cell disruption, citric acid, lactic acid, and acetic acid in 2.5 M concentration were used and no acid was used as the control sample. The color of carotenoid samples with various organic acids showed different colors, ranging from light pink to pink as in Figure 1A.

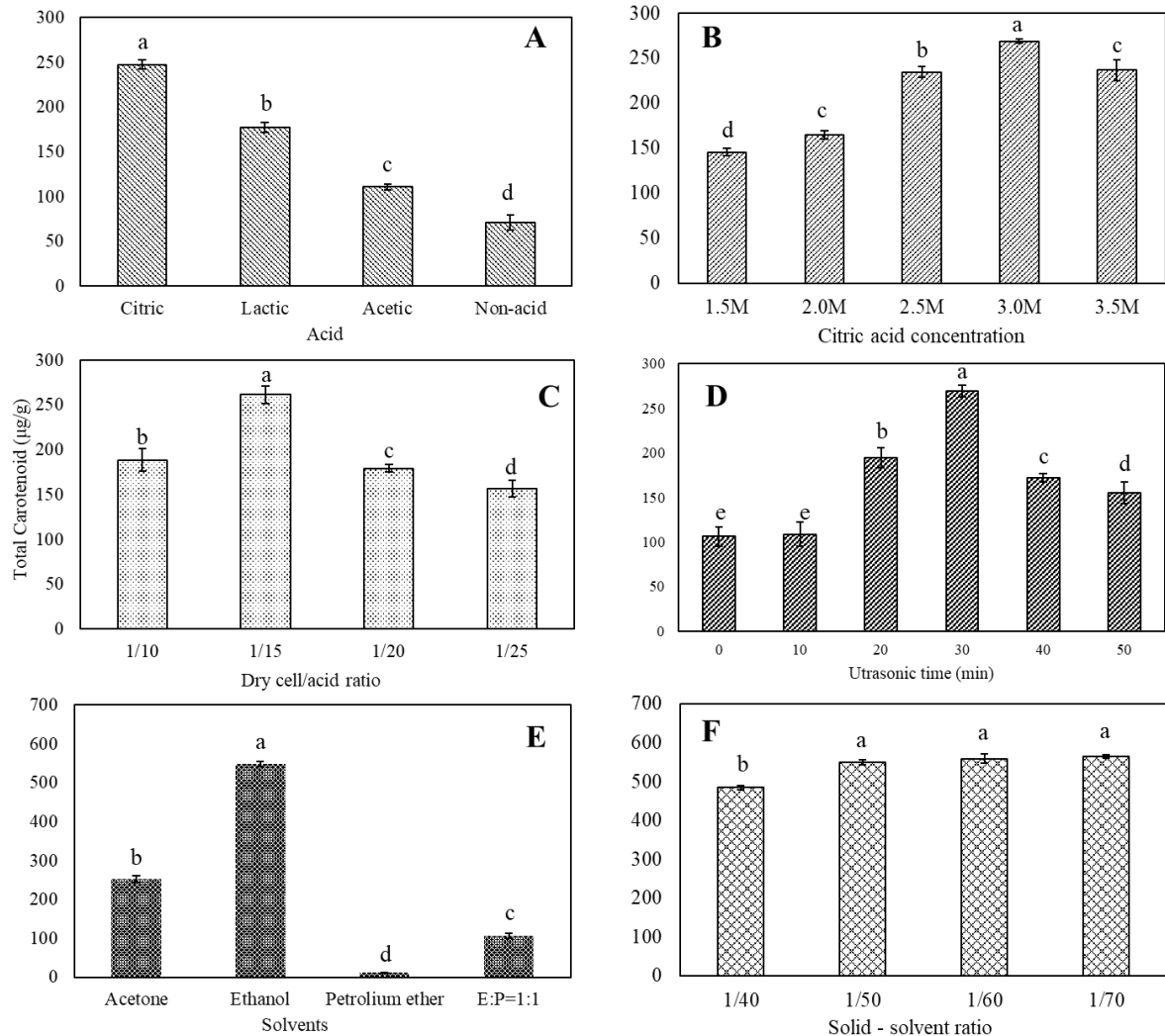


**Figure 1.** The color of carotenoid production of *R. mucilaginosa* in the different extraction condition. **A.** Type of organic acid in 2.5 M; **B.** Citric acid concentration; **C.** Dry cell/citric acid ratio (w/v); **D.** Ultrasound time (min)

From citric acid to lactic acid, and acetic acid, the color of carotenoid samples was gradually getting lighter, and the citric acid has given the darkest color. This result is consistent with TCC of various acids in Figure 2A. Results in Figure 2A showed that using the acid led to receiving higher TCC when comparing acid and no-acid sample. It was suggested that the acid disruption was effective on the cell disruption. Among using acids, citric acid resulted in the highest TCC that was approximately 3 times higher than the TCC of the control sample ( $247.33 \pm 5.03 \mu\text{g/g}$  compared to  $70.67 \pm 8.38 \mu\text{g/g}$ ). Following was lactic acid then acetic acid with  $177.33 \pm 3.40 \mu\text{g/g}$ ,  $110.67 \pm 8.38 \mu\text{g/g}$  of TCC, respectively.

The extraction efficiency is not the same in the different acids, it might depend on the acidity of these acids. Acids with higher acidity will be more effective in breaking down yeast cells and have a higher ability to extract carotenoids and vice versa. The acidity of an acid could be measured and compared by

the value of the acid dissociation constant pKa. The lower the acid dissociation constant, the stronger the bond between the proton and the acid molecule, so the higher the acidity. Citric acid has the highest acidity with a pKa constant of 3.15 in the first carboxyl group and 4.77 in the second carboxyl group. Meanwhile, lactic acid has a lower acidity with a pKa constant of 3.86 and ascorbic acid has the lowest acidity with a pKa constant of 4.10. Among the organic acids commonly found in foods, citric acid is also the acid with the lowest pKa constant [23].



**Figure 2.** Total carotenoid content in the different extraction condition. **A.** Type of organic acid in 2.5 M; **B.** Citric acid concentration; **C.** Dry cell/acid ratio (w/v); **D.** Ultrasound time (min); **E.** Type of solvent; **F.** Solid-solvent ratio. Different letters indicate statistically significant differences ( $P \leq 0.05$ ).

### 3.2. Effect of acid concentration on carotenoid extraction

Citric acid with different concentrations ranges from 1.5 M to 3.5 M were studied the acid concentration on the carotenoid extraction. The color of carotenoid sample is shown in Figure 1B. The color of carotenoid sample became darker when enhancing the acid concentration from 1.5 M to 3 M. At 3.5 M citric acid concentration, the color of carotenoid sample was lighter than that in the 3 M citric acid. It meant that the carotenoid content reduced if acid concentration continued increasing.

The TCC in Figure 2B indicated the same results of carotenoid color, which is the gradually increasing TCC when intensifying acid concentration. However, the maximum acid concentration was only at 3 M. If continuing to rise the acid concentration to 3.5 M, the TCC tends to decrease. In 3 M of citric acid, the TCC was  $268.67 \pm 2.31 \mu\text{g/g}$ , while the TCC was down  $236.67 \pm 11.37 \mu\text{g/g}$  in 3.5 M citric acid. The acidity of the environment depends on the acid concentration of the environment. The environment becomes more acidic and more efficient at dissolving cells as the concentration of acid

rises. The environment's acidity reaches its limit when the acid concentration reaches 3 M, so the resulting  $\beta$ -carotene content does not change at higher concentrations and tends to decrease as the acid concentration rises because acidic environments cause pigments to decompose [24].

### 3.3. Effect of dry cell/acid ratio on carotenoid extraction

The TCC of dry cell/acid ration experiment is shown in Figure 2C. When increasing the dry cell/acid ratio, the TCC increased from  $188.67 \pm 12.22 \mu\text{g/g}$  at a ratio of 1/10 g/mL to  $261.33 \pm 9.57 \mu\text{g/g}$  at a ratio of 1/15 g/mL. Due to the increased dry cell /acid ratio, the amount of acid in the medium increased, leading to higher cell lysis efficiency and carotenoid extraction. When continuing to increase the dry cell/acid ratio to 1/20 and 1/25 g/mL, the TCC was downed to  $179.33 \pm 4.11 \mu\text{g/g}$  at the ratio 1/20 g/mL and still  $156.67 \pm 8.99 \mu\text{g/g}$  at the ratio 1/25 g/mL. The reason is because the increased volume causes the cell density per medium volume to decrease, the ability to collide between cells during the ultrasound process decreases, leading to unsatisfactory cell disruption during the ultrasound process. Effectively, the resulting TCC is significantly reduced. From there, it could be encouraged that the dry cell/acid ratio not only affects the cell breakdown process but also has a certain influence on other factors related to the carotenoid extraction process. It is necessary to determine the optimal ratio to achieve high efficiency and influence other factors.

### 3.4. Effect of ultrasound time on carotenoid extraction

To investigate the effect of ultrasound time on the efficiency of carotenoid extraction from yeast, ultrasound in a time range from 0 to 50 min with jumps of 10 min were carried out. The color of carotenoid sample is shown in Figure 1D. The TCC is shown in Figure 2D.

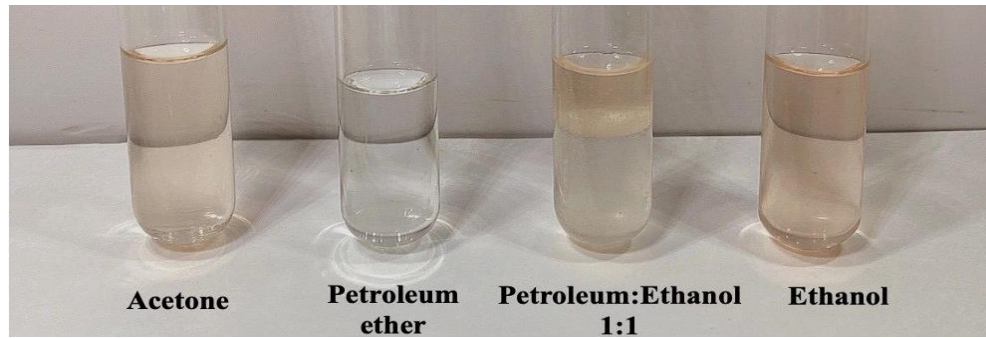
From these results, it could be suggested that ultrasound has a great impact on the efficiency of carotenoid extraction. The time of using ultrasound also greatly affects the TCC. During the first 10 min of ultrasound, the TCC was  $108.67 \pm 13.30 \mu\text{g/g}$  and no significant difference when compared with no ultrasound-assistant. However, after 20 min ultrasound, the TCC was  $194.67 \pm 11.12 \mu\text{g/g}$  that approximately 2 times higher than no ultrasound-assistant. The highest amount of TCC was recorded with 30 min of sonication, with the resulting being  $269.33 \pm 56.80 \mu\text{g/g}$ . When the ultrasonic treatment was settled longer than 30 minutes, the amount of TCC was decreased. At 40 min and 50 min ultrasound time, the TCC obtained only  $172.00 \pm 4.9 \mu\text{g/g}$  and  $155.33 \pm 12.26 \mu\text{g/g}$ , respectively.

According to Ye et al, ultrasound effectively disrupts the cell disruption process because it has the ability to create a turbulent flow within the liquid. During this process, particles in the liquid collide with each other at high speed [25]. Similarly, a study on ultrasonic also showed convective effects created by the continuous production and destruction of bubbles, further increasing the fluctuations of liquid caused by the transmission of ultrasonic [26]. Thus, ultrasound plays an important role in breaking cells, creating conditions for solvents to penetrate and dissolve carotenoids. In the study of Ye et al., ultrasound time is also a parameter that affects extraction yield because of the ability to generate heat or create free radicals in the solvent [25].

In our experiment, the temperature of the sample during the ultrasound process was controlled at room temperature. Thus, the carotenoid content gradually decreases when we perform ultrasound for more than 30 minutes. The reason might be that under ultrasound conditions, the solvent has the ability to create free radicals. If this happens, the carotenoid structures will be destroyed in two different ways: first, the double bonds in the carotenoid will be oxygenated to form  $-\text{C}=\text{O}$ , and second, the chains of the carotenoid molecule will be broken down or polymerized.

### 3.5. Effect of solvents on carotenoid extraction

Aim to replace acetone solvent by green solvents, other solvents including ethanol, petroleum ether and ethanol: petroleum ratio 1:1 were studied for their effects on carotenoid extraction. In which, the acetone solvent sample is used as a control sample. Ethanol and petroleum ether were used to replace acetone in the extraction to obtain friendly products and more easily apply to the food. The color of carotenoid sample with various solvents is shown in Figure 3 and TCC is shown in Figure 2E.



**Figure 3.** The color of carotenoid production of *R. mucilaginosus* in the different solvents.

The color of solvents after extraction was shown in Figure 3 indicated that the color of the extracted solvent samples changes from colorless (transparent) to bright pink (pink). The sample has the darkest bright pink color when using ethanol solvent for the extraction process. Particularly for samples using petroleum ether solvent, it is almost colorless. And for samples using an Ethanol: Petroleum ether ratio of 1:1, there was layer separation. This can explain why carotenoid is highly soluble in ethanol but slightly soluble in petroleum ether, in addition, ethanol has a molecular mass of 46.07 g/Mol lower than the molecular mass of petroleum ether which is 82.2 g/Mol. Therefore, the upper pink layer is a carotenoid that is soluble in ethanol solvent and the slightly opaque layer below is a carotenoid that is very slightly soluble in petroleum ether. In summary, the above color change shows that in the extracted samples, the TCC is different. TCC in ethanol solvent is  $548.98 \pm 6.30 \mu\text{g/g}$ , the highest compared to other solvents used for extraction (Figure 2.E).

Theories on the solubility of carotenoids suggest that carotenoids are soluble in fats and in solvents such as acetone, ethanol, diethyl ether, and chloroform. Carotenoid belongs to the carotene group that dissolves well in non-polar solvents such as hexane, acetone, etc. However, our experimental results show the ability to extract carotenoid from non-polar solvents acetone is many times lower than the solvent with only one non-polar end, ethanol.

Currently, there is no comparative study on the ability to extract carotenoid from yeast using the four solvents used above. However, for microorganisms, especially yeast, most current studies use the solvent acetone. In the study of optimizing the extraction process of carotenoids from *Rhodobacter sphaeroides* by Zhenxin Gu and colleagues (2007), acetone solvent was also used to perform the carotenoids extraction process. Research by Šovljanski and colleagues (2022) showed that for the *R. mucilaginosus* strain, the maximum carotenoids content was obtained when using acetone as the extraction solvent. Some studies use ethanol in extracting carotenoids from microorganisms or plants as in the study of A. Surendran et al., Ye et al., Ni et al., Meinhardt-Wollweber et al., Fang Qu et al., [18], [20], [25], [27], [28].

Regarding our group's research, we aim to use environmentally friendly extraction solvents. And the results obtained showed that carotenoid was extracted with the highest content when using ethanol as a solvent at 100% concentration with the ratio of 1 g dry yeast cell biomass/50 mL ethanol. The results were obtained after many repeated experiments, so the team's results are objective, but further research is needed to clarify the effective extraction of carotenoid in ethanol solution.

### **3.6. Effect of dry cell-solvent ratio on carotenoid extraction**

The dry cell-ethanol solvent ratio ranged from 1/40, 1/50, 1/60 to 1/70 g/mL was surveyed. Results of Figure 2F indicate that the dry cell-solvent ratio is low, the ability to dissolve carotenoids into the solvent is low, leading to a lower carotenoid content. However, at the ratios of 1/50, 1/60 and 1/70 g/mL, respectively, the obtained TCC did not clearly differ. TCC obtained at ratios of 1/40, 1/50, 1/60 and 1/70 ratio is  $484.48 \pm 5.19 \mu\text{g/g}$ ,  $548.98 \pm 6.30 \mu\text{g/g}$ ,  $558.78 \pm 11.37 \mu\text{g/g}$ ,  $563.74 \pm 4.36 \mu\text{g/g}$ , respectively with no statistically significant differences.

The essence of the extraction process is molecular diffusion. When the difference in carotenoid concentration in micelles (a mixture of solvent and carotenoids) and in dry cells is higher, the diffusion process is strong, diffusion occurs until reaching a state of equilibrium then stops. When too little solvent is used, the extraction efficiency is low because the solvent is not enough to dissolve the amount of

carotenoid in dry cells. However, if excess solvent is used, it would cause waste of solvent, and increase the amount of impurities, so the economic efficiency of the production process is invalid. Therefore, it is necessary to research the appropriate solvent-dry cell weight ratio to extract the maximum amount of carotenoids in dry cells and achieve the highest economic efficiency.

To both save solvent costs and still ensure high extraction efficiency, most appropriate ratio of dry cells/solvent for the extraction of carotenoids from the yeast strain *R. mucilaginosa* to be 1 g dry cells/50 mL solvent was chosen.

#### 4. Conclusions

After surveying the factors to extract carotenoids from yeast for application in food, the extraction conditions were established. The total carotenoid obtained is approximately 550 µg/g. Carotenoid in ethanol is a reliable supply of ingredients for food.

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#### Conflict of Interest

The authors declare no conflict of interest.

#### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

#### REFERENCES

- [1] V. Perrier, E. Dubreucq, and P. Galzy, "Fatty acid and carotenoid composition of *Rhodotorula* strains," *Arch. Microbiol.*, vol. 164, no. 3, pp. 173–179, 1995, doi: 10.1007/s002030050251.
- [2] T. Maoka, "Carotenoids as natural functional pigments," *J. Nat. Med.*, vol. 74, no. 1, pp. 1–16, 2020, doi: 10.1007/s11418-019-01364-x.
- [3] H. Aoki, N. T. Minh Kieu, N. Kuze, K. Tomisaka, and N. Van Chuyen, "Carotenoid pigments in gac fruit (*Momordica cochinchinensis* SPRENG)," *Biosci. Biotechnol. Biochem.*, vol. 66, no. 11, pp. 2479–2482, 2002, doi: 10.1271/bbb.66.2479.
- [4] H. Müller, "Determination of the carotenoid content in selected vegetables and fruit by HPLC and photodiode array detection," *Eur. Food Res. Technol.*, vol. 204, no. 2, pp. 88–94, 1997, doi: 10.1007/s002170050042.
- [5] J. Gross, *Pigments in Vegetables: Chlorophylls and Carotenoids*. 2002.
- [6] E. Yara-Varón, Y. Li, M. Balcells, R. Canela-Garayoa, A. S. Fabiano-Tixier, and F. Chemat, "Vegetable oils as alternative solvents for green oleo-extraction, purification and formulation of food and natural products," *Molecules*, vol. 22, no. 9, pp. 1–24, 2017, doi: 10.3390/molecules22091474.
- [7] A. Ludwiczuk, K. Skalicka-Woźniak, and M. I. Georgiev, "Terpenoids," in *In Pharmacognosy: Fundamentals, Applications and Strategy*, 2017, pp. 233–266. doi: 10.1016/B978-0-12-802104-0.00011-1.
- [8] G. Britton, *Carotenoids Handbook*, 1st ed. Birkhäuser Basel, 2004. doi: 10.1007/978-3-0348-7836-4.
- [9] K. Fliieger, N. Knabe, and J. Toepel, "Development of an improved carotenoid extraction method to characterize the carotenoid composition under oxidative stress and cold temperature in the rock inhabiting fungus *Knufia petricola* A95," *J. Fungi*, vol. 4, no. 4, 2018, doi: 10.3390/jof4040124.
- [10] I. R. Maldonado, D. B. Rodriguez-Amaya, and A. R. P. Scamparini, "Carotenoids of yeasts isolated from the Brazilian ecosystem," *Food Chem.*, vol. 107, no. 1, pp. 145–150, 2008, doi: 10.1016/j.foodchem.2007.07.075.
- [11] E. Biehler, F. Mayer, L. Hoffmann, E. Krause, and T. Bohn, "Comparison of 3 spectrophotometric methods for carotenoid determination in frequently consumed fruits and vegetables," *J. Food Sci.*, vol. 75, no. 1, 2010, doi: 10.1111/j.1750-3841.2009.01417.x.
- [12] P. Gupta, Y. Sreelakshmi, and R. Sharma, "A rapid and sensitive method for determination of carotenoids in plant tissues by high performance liquid chromatography," *Plant Methods*, vol. 11, no. 1, pp. 1–12, 2015, doi: 10.1186/s13007-015-0051-0.
- [13] M. Orosa, D. Franqueira, A. Cid, and J. Abalde, "Analysis and enhancement of astaxanthin accumulation in *Haematococcus pluvialis*," *Bioresour. Technol.*, vol. 96, no. 3, pp. 373–378, 2005, doi: 10.1016/j.biortech.2004.04.006.
- [14] J. K. Kim, J. I. Kim, N. K. Lee, Y. T. Hahm, M. Y. Baik, and B. Y. Kim, "Extraction of β-carotene produced from yeast *Rhodospiridium* sp. and its heat stability," *Food Sci. Biotechnol.*, vol. 19, no. 1, pp. 263–266, 2010, doi: 10.1007/s10068-010-0038-6.
- [15] D. B. Rodriguez-Amaya and M. Kimura, *HarvestPlus Handbook for Carotenoid Analysis*. 2004. doi: 10.3141/2068-08.
- [16] M. D. Macías, C. Mantell, M. Rodríguez, E. J. M. de la Ossa, L. M. Lubián, and O. Montero, "Supercritical Fluid Extraction of carotenoids from *Nannochloropsis Gaditana*," *Chem. Eng. Trans.* 2, no. 1, pp. 247–252, 2002.
- [17] M. Herrero, L. Jaime, P. J. Martín-Álvarez, A. Cifuentes, and E. Ibáñez, "Optimization of the extraction of antioxidants from *Dunaliella salina* microalga by pressurized liquids," *J. Agric. Food Chem.*, vol. 54, no. 15, pp. 5597–5603, 2006, doi: 10.1021/jf060546q.
- [18] A. Surendran, K. Sudha, B. Murugan, and R. Narayanan, "Extraction of Carotenoids from Carrot and Pumpkin using different Solvent Proportions," *Biological Forum-An International Journal*, vol. 14, no. 2, p. 582, 2022. [Online]. Available: [www.researchtrend.net](http://www.researchtrend.net)

- [19] N. Urano, A. Shirao, Y. Naito, M. Okai, M. Ishida, and M. Takashio, "Molecular Phylogeny and Phenotypic Characterization of Yeasts with a Broad Range of pH Tolerance Isolated from Natural Aquatic Environments," *Adv. Microbiol.*, vol. 09, no. 01, pp. 56–73, 2019, doi: 10.4236/aim.2019.91005.
- [20] H. Ni, Q. Chen, G. He, G. Wu, and Y. Yang, "Optimization of acidic extraction of astaxanthin from *Phaffia rhodozyma*," *J. Zhejiang Univ. Sci. B*, vol. 9, no. 1, pp. 51–59, 2008, doi: 10.1631/jzus.B061261.
- [21] L. Urnau, R. Colet, V. F. Soares, E. Franceschi, E. Valduga, and C. Steffens, "Extraction of carotenoids from *Xanthophyllomyces dendrorhous* using ultrasound-assisted and chemical cell disruption methods," *Can. J. Chem. Eng.*, vol. 96, no. 6, pp. 1377–1381, 2018, doi: 10.1002/cjce.23046.
- [22] H. K. Lichtenthaler, "Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes," *Methods Enzymol.*, vol. 148, no. C, pp. 350–382, 1987, doi: 10.1016/0076-6879(87)48036-1.
- [23] A. M. N. Silva, X. Kong, and R. C. Hider, "Determination of the pKa value of the hydroxyl group in the  $\alpha$ -hydroxycarboxylates citrate, malate and lactate by <sup>13</sup>C NMR: Implications for metal coordination in biological systems," *BioMetals*, vol. 22, no. 5, pp. 771–778, 2009.
- [24] E. Günerken, E. D. Hondt, M. H. M. Eppink, L. Garcia-gonzalez, K. Elst, and R. H. Wijffels, "Cell disruption for microalgae biorefineries," vol. 33, pp. 243–260, 2015, doi: 10.1016/j.biotechadv.2015.01.008.
- [25] J. Ye, L. Feng, J. Xiong, and Y. Xiong, "Ultrasound-assisted extraction of corn carotenoids in ethanol," *Int. J. Food Sci. Technol.*, vol. 46, pp. 2131–2136, 2011, doi: 10.1111/j.1365-2621.2011.02727.x.
- [26] Y. Liu, X. Liu, Y. Cui, and W. Yuan, "Ultrasound for microalgal cell disruption and product extraction: A review," *Ultrason. Sonochem.*, vol. 87, no. February, p. 106054, 2022, doi: 10.1016/j.ultsonch.2022.106054.
- [27] M. Meinhardt-Wollweber, C. Suhr, A. K. Kniggendorf, and B. Roth, "Absorption and resonance Raman characteristics of  $\beta$ -carotene in water-ethanol mixtures, emulsion and hydrogel," *AIP Advances*, vol. 8, no. 5, 2018, doi: 10.1063/1.5025788.
- [28] F. Qu *et al.*, "Temperature effect on electronic and vibrational properties of  $\beta$ -carotene aggregates in aqueous ethanol solution," *Dyes and Pigments*, vol. 166, pp. 323–329, 2019, doi: 10.1016/j.dyepig.2019.03.049.



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