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Research Article

# High-density cultivation of *Phaffia rhodozyma* SFAS-TZ08 in sweet potato juice for astaxanthin production

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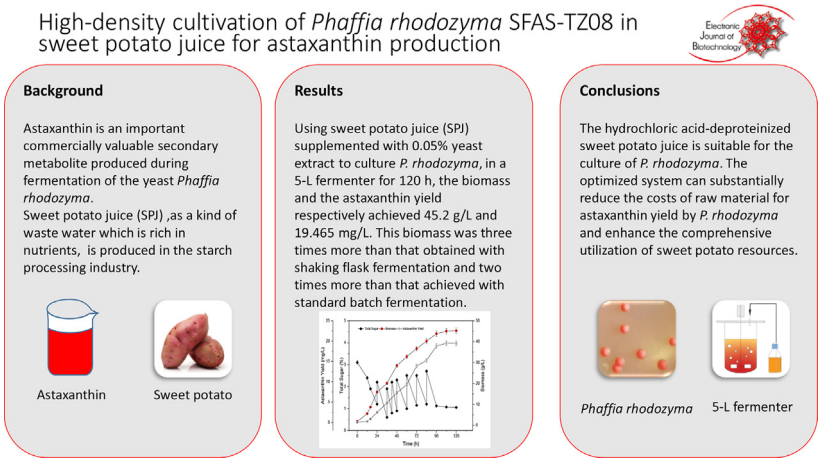
## GRAPHICAL ABSTRACT

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## ABSTRACT

*Background:* Astaxanthin is an important commercially valuable secondary metabolite produced during fermentation of the yeast *Phaffia rhodozyma* that can be used in aquatic feedstock.
*Results:* In this study, we used sweet potato juice (SPJ), the by-product of sweet potato starch, to obtain a high-density culture of *P. rhodozyma*. We confirmed that hydrochloric acid deproteinized SPJ is suitable for the culture of *P. rhodozyma*, and using this as a substrate, supplemented with 0.05% yeast extract, we performed batch fermentation in a 5 L fermenter. Compared to shaking flask fermentation, we obtained an 18.86% increase in yeast biomass and a 32.5% increase in astaxanthin yield using the batch process. After culturing *P. rhodozyma* in a 5 L fermenter for 120 h, we achieved biomass and astaxanthin yield of 45.2 g/L and 19.465 mg/L, respectively.
*Conclusions:* In this study, we found that deproteinized SPJ was the most suitable for *P. rhodozyma* through experiments on different cultivars and different processing stages of SPJ. On the basis of deproteinized SPJ, supplemented with yeast extract, the biomass and astaxanthin yield reached a high level.

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*Phaffia rhodozyma*
Sweet potato juice (SPJ)
Unsaturated terpenoid
Yeast

The optimized system can substantially reduce the costs of raw material for astaxanthin yield by *P. rhodozyma* and enhance the comprehensive utilization of sweet potato resources.

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## 1. Introduction

Astaxanthin (3,3′-dihydroxy-β, β'-carotene-4,4′-dione) is an unsaturated terpenoid compound consisting of a long-conjugated double-bonded carbon chain with four isoprene structures and six-membered rings of α-hydroxy ketones at each end [1], which is structurally similar to β-carotenes [2]. Astaxanthin is an extremely powerful antioxidant, and it has the ability to quench singlet oxygen and trap free radicals. Indeed, of the more than 600 identified natural carotenoids, astaxanthin is considered as the most powerful antioxidant, being 500 and 38 times more potent than vitamin E and β-carotene, respectively [3]. The antioxidant capacity of astaxanthin protects cells, lipids, and membrane lipoproteins from oxidative damage [4], and in addition, it has also been established to have a diverse range of other biological properties, including anti-inflammatory [5] and anticancer [6,7] activities, vision improvement [8], and immune system enhancement [9].

Methods for the production of astaxanthin can be divided into two main categories: chemical synthesis and extraction. Chemically synthesized astaxanthin is a mixture of three stereospecific structures with low antioxidant activity, and the Food and Drug Administration (FDA) has banned the inclusion of chemically synthesized astaxanthin in healthy food products. The production of astaxanthin via extraction can be further divided into three types according to its source, namely, aquatic animals, yeast, and microalgae. The extraction of astaxanthin from aquatic animals such as shrimps and crabs is a complex process, and given the paucity of discarded shrimps and crabs, along with the associated high extraction costs and serious pollution, the benefits of this method are somewhat limited. Although the microalga *Haematococcus pluvialis* is known for its high cellular astaxanthin content, it tends to have demanding growth conditions, on account of prolonged culture cycles and complex process controls. In contrast, the culture of *Phaffia rhodozyma* is comparatively straight forward and requires relatively basic raw materials, such as sucrose and glucose, to produce astaxanthin. Moreover, the rate of cell reproduction is adequate, and process controls are simple. However, the yield of astaxanthin obtained from *P. rhodozyma* tends to be low, and the cost of production from sucrose is relatively high. Sucrose can promote the increase of biomass and is one of the common carbon sources for the cultivation of *P. rhodozyma*. To reduce the costs of raw materials for the production of astaxanthin by *P. rhodozyma*, numerous studies have evaluated the use of alternative less expensive raw materials for fermentation, such as the direct use of raw plant materials, including *Yucca filifera* date juice [10], sweet sorghum juice [11,12], and sugarcane juice [13]; waste from the food and wood processing industries; waste liquids; and even food waste [14]. The use of industrial wastes as a substrate not only reduces the cost of raw materials but also resolves the problems of waste disposal and pollutant discharges.

Sweet potato, a starch-rich crop grown worldwide [15], is a staple food in African countries such as Uganda and Burundi. In China, a variety of sweet potatoes are used to produce starch. Sweet potatoes are pulped with water, and the residues are separated by centrifugation to obtain starch. Large volumes of waste water were generated during this process, most of them were consisted of sweet potato juice (SPJ). SPJ has a total sugar content of approximately 3-4% with sucrose, fructose, and glucose accounting for approximately 60% of total sugars, in addition to a small amount of protein and a variety of vitamins and minerals in SPJ. To date, studies on the utilization of SPJ resources have included the extraction of sweet potato storage proteins [16], polyphenol oxidase [17], and β-amylase [18]. Because of the high cost of isolation, most of these works remain at the laboratory stage. SPJ has also been used as a microbial fermentation medium to culture *Bacillus saprophyticus* as a biopesticide [19] and to produce biodiesel [20].

In this work, SPJ was used as an inexpensive raw material for culturing the *P. rhodozyma* for the first time, which not only reduced the fermentation cost but also realized the utilization of sweet potato resources in all aspects.

## 2. Materials and methods

### 2.1. Materials

Hydrochloric acid, sodium hydroxide, citric acid, glucose, and acetone were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), all of which were of analytical grade. Fresh sweet potato of the varieties Jishu 25, Jishu 26, Yanshu 25, Shangshu 19, and Xushu 18 were provided by the Shandong Academy of Agriculture, among which Yanshu 25 and Jishu 26 are edible cultivars, and Jishu 25, Xushu 18, and Shangshu 19 are used to produce starch.

### 2.2. Preparation of SPJ

SPJ was prepared as indicated in Fig. 1, which shows a schematic representation of the sweet potato starch production processes. Specifically, fresh sweet potato tubers were cleaned and divided into 1 cm × 1 cm sections, to which was added 2.5 times the volumes of water, followed by grinding using an Y912 cell wall disruptor (Joyoung, Shandong, China). The ground material was subsequently filtered through a 200 mesh filter cloth to obtain starch pulp, which was then centrifuged for 1 min at 3,000 r/min in an L530 centrifuge (Cence, Hunan, China), and the supernatant was collected as SPJ-A. SPJ-A was heated to 50°C, after which, the pH was adjusted to 4.0 using 5% hydrochloric acid, and the suspension was allowed to stand for 2 h prior to centrifugation at 10,000 *g* for 15 min in a CR15RE high-speed refrigerated centrifuge (Hitachi, Chiyoda, Japan). The supernatant was collected as SPJ-B. Similarly, SPJ-A was heated to 50°C, adjusted to pH 4.0 using citric acid, allowed to stand for 2 h, and then centrifuged for 15 min at 8,000 r/min in a CR15RE high-speed refrigerated centrifuge. The resulting supernatant was collected as SPJ-D. To obtain SPJ-C, SPJ-B was evaporated using a SY-5000 rotary evaporator (Yarong, Shanghai, China) at 150 mbar to yield a solid preparation of 50% juice concentrate. To ensure accuracy of the experimental data, experiments using the juices obtained from the different sweet potato cultivars were conducted using the same process. All other experiments were conducted using SPJ prepared from the same

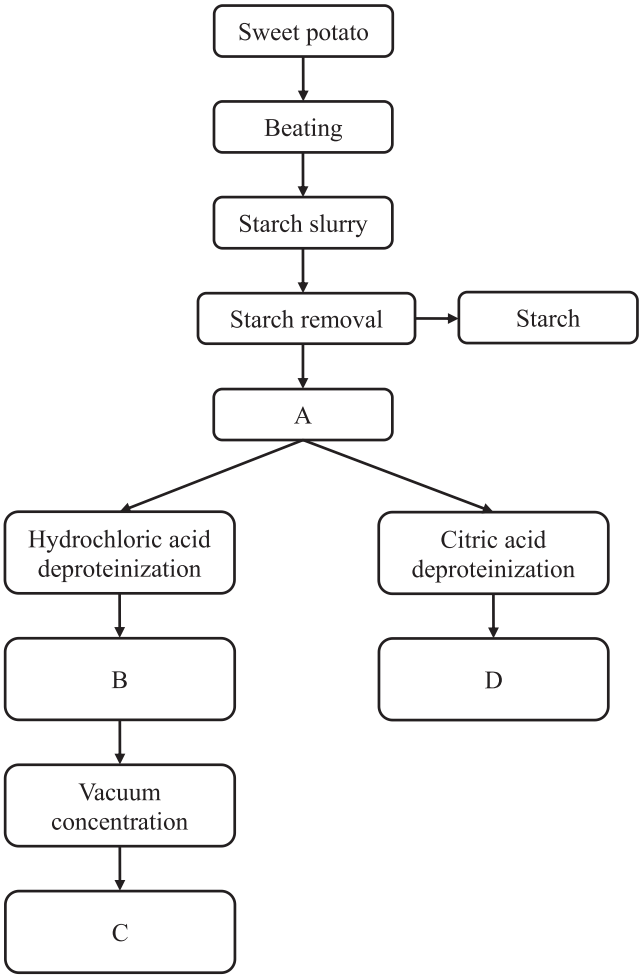


**Fig. 1.** A flow chart of SPJ preparation.

batch of Jishu 25, and the concentration of SPJ was based on the concentration of the soluble solid contents.

### 2.3. Microorganisms and inoculum preparation

#### 2.3.1. Preparation of inoculum

*P. rhodozyma* SFAS-TZ08 was provided by the Shandong Food Fermentation Industry Research and Design Institute (Jinan, China) and cultured in yeast malt (YM) medium, comprising yeast extract (0.3%), peptone (0.5%), and glucose (2.0%). Yeast strains were used to inoculate 50 mL of sterilized YM medium contained in 500 mL flasks, which were incubated at 20°C for 24 h. *P. rhodozyma* inoculated at 5% (v/v) was transferred to fresh YM medium for 16 h.

#### 2.3.2. Shake flask cultivation

For culturing the control group, we used a composite medium (CM) containing 4% sucrose and 0.3% yeast extract, and to investigate the suitability of SPJ as a substrate for *P. rhodozyma* growth, we performed a shaking batch evaluation. *P. rhodozyma* inoculated at 5% (v/v) was cultured in 500 mL flasks containing 50 mL of liquid medium at 20°C, pH 5.0, and 200 r/min rotary agitation.

#### 2.3.3. Bioreactor cultivation

Bioreactor experiments were conducted in 5 L glass fermenters with a total volume of 3.5 L. As a substrate, we used autoclaved (118°C for 30 min) SPJ supplemented with 0.05% yeast extract at an initial pH of 5.0, which having been cooled to 20°C was inoculated with 5% seed culture. The culture was then incubated for 120 h, temperature was controlled at 20°C, the air flow rate was set at 4.0 L/min, and the stirring speed was adjusted to 400 r/min. A 12 h intervals, samples were collected and frozen for subsequent use. Experimental trials were conducted in duplicate. Batch replenishment fermentation was performed using essentially the same procedure as that used for standard batch fermentation, though with additional replenishment of 100 mL of SPJ-C at 0.5–1% of the total sugar remaining. In addition, samples were taken before and after replenishment and frozen for subsequent use. Experimental evaluations were performed in triplicate.

### 2.4. Analytical methods

#### 2.4.1. Biomass detection

The yeast biomass generated was determined by centrifuging 10 mL of fermentation broth at 5,000 r/min for 10 min, rinsing three times with distilled water, clarifying the washing solution, drying at 80°C for 15 h, and finally drying to a constant weight. Biomass was calculated on a dry weight (g/L) basis [21].

#### 2.4.2. Extraction and quantification of astaxanthin

Adjustments were made as described previously by Yin et al. [22]. Aliquots (5 mL) of the culture solution was centrifuged for 15 min at 8,000 r/min, washed three times, and mixed with 3 mL of 3 mol/L HCl. The resulting solution was boiled in a water bath for 3 min, rapidly cooled, centrifuged, and again washed three times. Thereafter, 5 mL of acetone was added, and following shaking for 1 min, a supernatant was obtained via centrifugation. The optical density of this preparation was measured at 476 nm using a Unico 7200 spectrophotometer (Shanghai, China). A calibration curve was prepared using different concentrations of astaxanthin, with acetone used as a blank.

#### 2.4.3. Determination of total sugar content

Total sugar content was determined colorimetrically using Felling's reagent [23]. SPJ was centrifuged in a CR15RE centrifuge at 5,000 r/min for 10 min, and to 20 mL of the resulting supernatant, we added 20 mL of concentrated HCl using water to adjust the total volume to 100 mL. Of this, after heating at 121°C for 30 min and after cooling to 25°C, 20% NaOH was added to neutralize the pH and adjust the volume to 200 mL. The resulting solution was then titrated against a 1 g/L solution of glucose standard.

#### 2.4.4. Data calculation and analysis

The astaxanthin conversion rate ($Y_{G/S}$) represents the ratio of astaxanthin concentration to that of the total sugar consumed, whereas the biomass conversion rate ($Y_{M/S}$) is the ratio of biomass concentration to that of the total sugar consumed. The astaxanthin and biomass conversion rates were calculated using Equations 1 and 2, respectively:

$$Y_{G/S} = \frac{C_G}{C_S} \quad (1)$$

$$Y_{M/S} = \frac{C_M}{C_S} \quad (2)$$

where $C_G$ is the astaxanthin concentration, $C_M$ is the biomass concentration, and $C_S$ is the total sugar content. $Y_{G/S}$ is the astaxanthin conversion rate, and $Y_{M/S}$ is the biomass conversion rate.

Cellular astaxanthin content, which is the ratio of astaxanthin concentration to that of biomass, was determined using Equation 3:

$$Y_{G/M} = \frac{C_G}{C_M} \quad (3)$$

*2.4.5. Determination of soluble solids (SS)*

The soluble solid contents of both SPJ fermentation broth and SPJ samples were determined from the respective supernatants obtained by centrifugation at 5,000 r/min for 10 min in a CR15RE centrifuge, and determinations were performed using a PAL-1 refractometer for soluble solids (ATAGO, Tokyo, Japan) [24].

*2.4.6. Analysis of samples containing sucrose, glucose, and fructose*

Sugars were detected using a Thermo UltiMate 3000 series HPLC system (Waltham, MA, USA) equipped with a Waters Sugar-Pak I column (Milford, MA, USA), based on refractive index (RI) detection. The mobile phase was ultrapure water obtained using an Aisrv-7 water purifier (Aisrv, Jiangsu, China), which was filtered through a 0.2 μm nylon microporous membrane (Tianjin, China) prior to use. For each HPLC run, standard samples containing sucrose, glucose, and fructose were also analyzed.

SPJ samples were heated to 50°C, cooled to room temperature for 10 min, and centrifuged at 10,000 r/min for 10 min. The supernatant thus obtained was subsequently syringe filtered through a 0.2 μm nylon filter into sample bottles in preparation for sampling. Similarly, 10 mL aliquots of fermentation broth collected prior to and after fermentation were centrifuged at 5,000 r/min for 10 min, and the resulting supernatants were syringe filtered through a 0.2 μm nylon filter into sample bottles for the determination of sucrose, glucose, and fructose contents in the fermentation broth.

*2.4.7. Determination of protein*

The protein content of SPJ was determined using the Kjeldahl method [25].

*2.5. Statistical Analysis*

Experiments were performed in triplicate, and the results are presented as mean ± 95% confidence interval. One-way analysis of variance (ANOVA) performed in conjunction with Tukey's test was used to evaluate the statistical significance of differences between mean values. Differences were considered statistically significant at the $P < 0.05$ level.

## 3. Results

*3.1. The effect of sweet potato cultivars*

In this study, we assessed the astaxanthin production of *P. rhodozyma* cultured using SPL obtained from five selected sweet potato cultivars, namely, Yanshu 25 and Jishu 26 (edible cultivars) and Jishu 25, Xushu 18, and Shangshu 19 (starch processing cultivars). Edible cultivars of sweet potato contain 20–30% dry matter, of which approximately 5–10% is soluble sugar, 30–50% is starch, and 1–3% is protein. In comparison, cultivars used for starch processing contain 30–35% dry matter, of which approximately 2–5% is soluble sugar, 50–80% is starch, and 4–9% is protein [26]. We then determined the suitability of SPJ prepared from different sweet potato cultivars as a medium for astaxanthin production by *P. rhodozyma*.

The results (Table 1) revealed that at the same SS concentration, the biomass of *P. rhodozyma* produced with SPJ obtained from edible cultivars was higher than the starchy cultivars, with the highest and lowest biomasses of 15.67 and 13.19 g/L, with SPJs of Yanshu 25 and Shangshu 19, respectively. We speculate that the differences in yield can be attributed primarily to differences in total sugar content among the SPJs of the different cultivars, with those in the edible cultivars being higher (reaching 3.89% for Yanshu 25) than those in the starchy cultivars, thereby indicating that an available carbon source is favorable for biomass accumulation. Moreover, we detected similar results in astaxanthin yield and biomass, which were positively correlated with total sugar content in the SPJs. However, the astaxanthin conversion rate ($Y_{G/S}$), biomass conversion rate ($Y_{M/S}$), and cellular astaxanthin content ($Y_{G/M}$) tended to be more favorable when using starchy variety SPJs. Among the five cultivars, the SPJ of Jishu 25 was found to have the highest $Y_{G/M}$ value, indicating that the SPJ of this variety would be particularly conducive to the synthesis of astaxanthin. Therefore, we used Jishu 25 SPJ as a raw material substrate to culture *P. rhodozyma* for astaxanthin yield in the subsequent experiments.

*3.2. The effect of the SPJ preparation process*

For the purpose of this study, we prepared four different SPJs (A, B, C, and D), as described in Section 2.2, using the fresh tubers of Jishu 25 as the starting material. The soluble solids, contents of sugar and protein in SPJs (A, B, C, and D) are listed in Table 2. Using SPJ-A, SPJ-B, SPJ-C, and SPJ-D as culture medium (CM), the effects of SPJ and deproteinized SPJ on the growth and astaxanthin yield of *P. rhodozyma* were mainly studied, and the CM was used as the control.

As shown in Table 3, the biomass of *P. rhodozyma* obtained using SPJ-A (13.73 g/L) was higher than that obtained when using CM (13.40 g/L). SPJ is a good material to culture *P. rhodozyma*. The yields of astaxanthin obtained using SPJ-B (4.852 mg/L) and SPJ-D (4.819 mg/L) were higher than those using SPJ-A (4.605 mg/L), thereby indicating that removing the sweet potato protein from SPJ-A could enhance the growth of *P. rhodozyma* and increase the yield of astaxanthin. Moreover, we detected almost no difference in G obtained using SPJ-B and SPJ-C (4.846 mg/L), indicating that using either fresh or concentrated SPJ would have a significant effect on strain growth. Consequently, these findings indicate that SPJ-C could be used as a viable alternative of expensive semisynthetic nutrients in culturing *P. rhodozyma* for astaxanthin yield.

*3.3. The effect of sweet potato juice concentration*

To assess the effects of SPJ concentration on the biomass, astaxanthin yield, and cellular astaxanthin content of *P. rhodozyma*, we cultured yeast in medium containing different concentrations of SS

**Table 1**
Effect of different cultivars of SPJ on the fermentation of *P. rhodozyma.*

| Varieties | | TS (%) | M (g/L) | G (mg/L) | $Y_{G/S}$ (mg/g) | $Y_{M/S}$ (g/g) | $Y_{G/M}$ (mg/g) |
|---|---|---|---|---|---|---|---|
| Food | Jishu 26 | 3.36 ± 0.17$^{b}$ | 14.23 ± 0.71$^{b}$ | 4.815 ± 0.241$^{b}$ | 0.144 ± 0.007$^{c}$ | 0.424 ± 0.021$^{c}$ | 0.338 ± 0.017$^{c}$ |
| | Yanshu 25 | 3.89 ± 0.19$^{a}$ | 15.67 ± 0.78$^{a}$ | 4.960 ± 0.248$^{a}$ | 0.127 ± 0.006$^{d}$ | 0.402 ± 0.020$^{d}$ | 0.317 ± 0.016$^{d}$ |
| | Shangshu 19 | 2.99 ± 0.15$^{d}$ | 13.17 ± 0.66$^{d}$ | 4.535 ± 0.227$^{d}$ | 0.152 ± 0.008$^{b}$ | 0.440 ± 0.022$^{ab}$ | 0.344 ± 0.017$^{c}$ |
| Starch | Jishu 25 | 3.04 ± 0.15$^{c}$ | 13.40 ± 0.67$^{c}$ | 4.735 ± 0.237$^{c}$ | 0.156 ± 0.008$^{a}$ | 0.441 ± 0.022$^{a}$ | 0.353 ± 0.018$^{a}$ |
| | Xushu 18 | 2.97 ± 0.15$^{e}$ | 13.10 ± 0.66$^{e}$ | 4.568 ± 0.228$^{d}$ | 0.153 ± 0.008$^{b}$ | 0.440 ± 0.022$^{ab}$ | 0.349 ± 0.017$^{b}$ |

*TS: total sugar, M: biomass, G: astaxanthin yield, $Y_{G/S}$: astaxanthin conversion rate, $Y_{M/S}$: biomass conversion rate, $Y_{G/M}$: cellular astaxanthin content.
Different letters (a–e) represent significant differences among data in the same column ($P < 0.05$).

**Table 2**
The soluble solids, sugar and protein contents of sweet potato juice.

| SPJ | SS (%) | TS (%) | Sucrose (g/L) | Glucose (g/L) | Fructose (g/L) | Protein (g/L) |
|---|---|---|---|---|---|---|
| A | 4.1 | 3.32 ± 0.17$^{b}$ | 21.12 ± 0. 11$^{b}$ | 6.28 ± 0.03$^{b}$ | 5.86 ± 0.03$^{b}$ | 10.40 ± 0.05$^{b}$ |
| B | 4.0 | 3.30 ± 0.17$^{b}$ | 21.05 ± 0.11$^{c}$ | 6.26 ± 0.03$^{c}$ | 5.68 ± 0.03$^{c}$ | 1.93 ± 0.01$^{c}$ |
| C | 50.0 | 41.20 ± 2.06$^{a}$ | 263.01 ± 13.15$^{a}$ | 78.02 ± 3.90$^{a}$ | 71.03 ± 3.55$^{a}$ | 18.52 ± 0.93$^{a}$ |
| D | 4.0 | 3.29 ± 0.17$^{b}$ | 21.05 ± 0.11$^{c}$ | 6.25 ± 0.03$^{c}$ | 5.67 ± 0.03$^{c}$ | 1.92 ± 0.01$^{c}$ |

* SS: soluble solids, TS: total sugar, SPJ: sweet potato juice. A, B, C, and D are different SPJs shown in Figure 1.
Different letters (a–d) represent significant differences among the data in the same column ($P < 0.05$).

**Table 3**
*P. rhodozyma* cultivation performance in SPJ.

| SPJ | M (g/L) | G (mg/L) | $Y_{G/S}$ (mg/g) | $Y_{M/S}$ (g/g) | $Y_{G/M}$ (mg/g) |
|---|---|---|---|---|---|
| A | 13.73 ± 0.69$^{c}$ | 4.605 ± 0.230$^{d}$ | 0.138 ± 0.007$^{b}$ | 0.413 ± 0.021$^{c}$ | 0.335 ± 0.017$^{d}$ |
| B | 14.28 ± 0.71$^{a}$ | 4.852 ± 0.242$^{a}$ | 0.147 ± 0.007$^{a}$ | 0.433 ± 0.022$^{a}$ | 0.340 ± 0.017$^{c}$ |
| C | 14.28 ± 0.71$^{a}$ | 4.846 ± 0.241$^{a}$ | 0.147 ± 0.007$^{a}$ | 0.433 ± 0.022$^{a}$ | 0.339 ± 0.017$^{c}$ |
| D | 14.00 ± 0.70$^{b}$ | 4.819 ± 0.241$^{b}$ | 0.146 ± 0.007$^{a}$ | 0.425 ± 0.021$^{b}$ | 0.344 ± 0.017$^{b}$ |
| CM | 13.40 ± 0.67$^{d}$ | 4.713 ± 0.236$^{c}$ | 0.118 ± 0.006$^{c}$ | 0.335 ± 0.017$^{d}$ | 0.352 ± 0.018$^{a}$ |

* SPJ: sweet potato juice, M: biomass, G: astaxanthin yield, YG/S: astaxanthin conversion rate, YM/S: biomass conversion rate, YG/M: cellular astaxanthin content. A, B, C, and D are different SPJs see Figure 1.
Different letters (a–e) represent significant differences among the data in the same column ($P < 0.05$).

substrate, prepared by diluting SPJ-C preparations with water. As shown in Fig. 2, the results revealed that cell biomass and G increased with the increase of SPJ concentration, with biomass and G peaking at a concentration of 8%, with values of 23.92 g/L and 7.151 mg/L, respectively. However, whereas the $Y_{G/M}$ value showed an initial increasing trend in response to an increase in SPJ concentration, and reached a maximal value of 0.344 mg/g at an SS proportion of 3%, it subsequently underwent a decline, indicating that SPJ with an SS content of 3% would be the most favorable for the synthesis of astaxanthin by *P. rhodozyma*.

### 3.4. The effect of yeast extract supplement

Yeast extract is a common organic nitrogen source used in the fermentation of *P. rhodozyma* [27], and in our previous experiment, we found that yeast extract was beneficial not only for biomass but also for cellular astaxanthin content (Fig. 3). Consequently, in the present study, we investigated the effects of yeast extract addition on the culture *P. rhodozyma,* and accordingly we found that yeast extract supplementation could promote both *P. rhodozyma* biomass and astaxanthin accumulation. Compared to the control, the biomass and astaxanthin yield values were 15.19% and 11.98% higher, respectively, when 0.5 g/L yeast extract was supplemented in the SPJ-C medium.

### 3.5. Bioreactor cultivation

#### 3.5.1. Batch fermentation

In general, higher biomass and yields can be obtained from fermenter than shaking flask [28]. In the present study, we cultured *P. rhodozyma* in a 5 L bioreactor using SPJ-C with a SS concentration of 3% (total sugar content 2.4%) and 0.5 g/L yeast extract supplementation, the incubation process of which is illustrated in Fig. 4. From 0 h to 36 h, we observed a rapid increase in the biomass of *P. rhodozyma*, concomitant with a rapid reduction in total sugar content in the medium. Comparatively, there was an initial lag in the production of astaxanthin, which nevertheless, subsequently accumulated rapidly from 24 h to 48 h. Given that astaxanthin is a secondary metabolite with a long metabolic pathway, this delay in intracellular synthesis is unsurprising. After 72 h of fermentation, there was no further significant increase in either biomass or G. Culturing *P. rhodozyma* via bioreactor fermentation obtained maximum biomass and G of 15.46 g/L and of 6.045 mg/L, respectively, with corresponding increases of 18.9% and 32.5% compared with that by shaking flask fermentation.

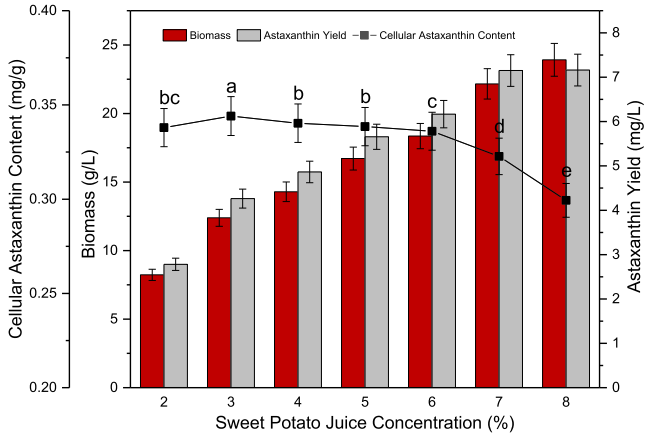


**Fig. 2.** Biomass, astaxanthin yield, and cellular astaxanthin content at different concentrations of SPJ-C.

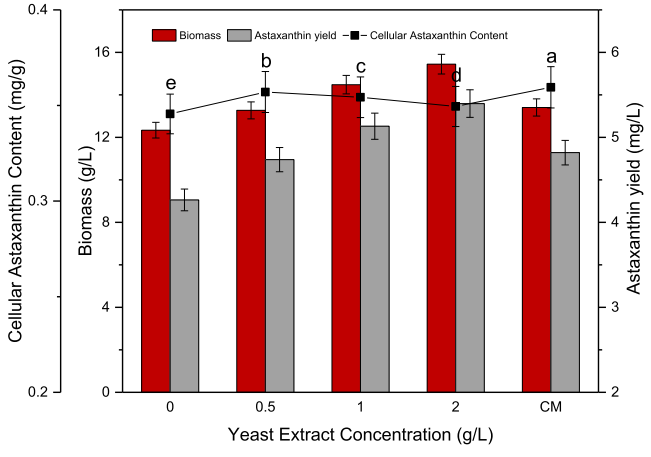


**Fig. 3.** Biomass, astaxanthin yield, and cellular astaxanthin content at different yeast extract concentrations.

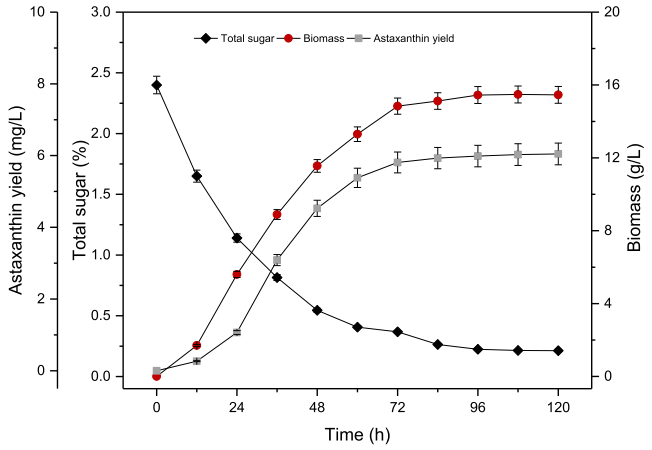


**Fig 4.** The batch fermentation process curve in a 5 L bioreactor.

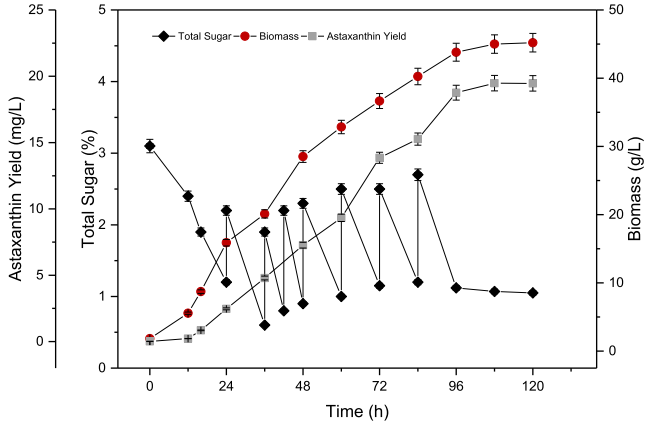


**Fig. 5.** Total sugar, biomass, and astaxanthin yield for SPJ cultures in a 5 L bioreactor.

*3.5.1.1. Fed-batch Fermentation.* To further enhance the astaxanthin yield, we used batch replenishment of high-density cultures of *P. rhodozyma*. Similar to the standard batch fermentation, a 3% SS SPJ-C supplemented with 0.5 g/L yeast extract was used as an initial medium. At 24 h after inoculation, SPJ-C containing 50% SS was batch-fed to the fermenter when the remaining total sugar was approximately 0.5–1%. SPJ-C was fed until a TS concentration of 2–2.5% was obtained, and supplementation was continued until 84 h, with fermentation being terminated at 120 h. Fig. 5 showed the 120 h profiles of total sugar consumption, biomass, and G obtained using this feeding strategy, which shows that a biomass of 45.20 g/L and G of 19.501 mg/L were obtained. This biomass was three times more than that obtained with shaking flask fermentation and two times more than that achieved with standard batch fermentation. Similarly, the astaxanthin yield using fed-batch fermentation was more than four times higher than that in shaking flask fermentation and three times higher than that in standard batch fermentation.

## 4. Discussion

Currently, *H. pluvialis* and *P. rhodozyma* are the main microorganisms used for astaxanthin production. Compared to *H. pluvialis*, *P. rhodozyma* can be heterotrophic cultivated with rich source of raw material, short culture period, and high biomass. However, the cellular astaxanthin content of *P. rhodozyma* is relatively low, resulting in a higher production cost for *P. rhodozyma* than for *H. pluvialis*. In addition to increasing the capacity of the *P. rhodozyma* itself to synthesize astaxanthin, the cost of astaxanthin production can be reduced by using low-cost raw materials and by obtaining higher biomass (CM optimization, extended cultures).

Previously, it has been shown that the addition of glucose and sucrose in synthetic medium can result in higher biomass, while yeast extract has been found to be conducive to higher astaxanthin yields (increased by 828.2 μg/L) [29]. To reduce the cost of raw materials, there have been attempts to produce astaxanthin directly from *P. rhodozyma* cultured from plant raw materials with high sucrose, glucose, and fructose content, such as sorghum juice, beet molasses, and Jerusalem artichoke. Stoklosa et al. [12] directly cultured *P. rhodozyma* in sorghum juice (12% total sugar concentration and 7.5% sucrose concentration) at a biomass of only 2.38 g/L. Then, the sorghum juice was supplemented with a 2.4% mixed nitrogen source (yeast extract and urea) for 168 h. The biomass reached a maximum of 28.8 g/L, a 10-fold increase over the synthetic medium. Jiang et al. [30] used Jerusalem artichoke (3% total sugar) supplemented with 8.6 g/L peptone, and the biomass reached 12.22 g/L after 108 h of batch fermentation; on top of this medium, 40% sucrose was added to the flow, and the fermentation was supplemented for 13 d. The final biomass reached 83.6 g/L. An et al. [31] used 1% sucrose synthesis medium as the initial medium and fermented for 180 h with feeding molasses (cumulative supplementation of 12% total sugar) to achieve a biomass of 36 g/L. In this study, the biomass of *P. rhodozyma* fermented with 3% SPJ reached 12.34 g/L, which was slightly lower than 13.41 g/L with CM (4% sucrose, 0.3% yeast extract). However, the addition of 0.5 g/L yeast extract increased the batch fermentation biomass by 18.9% to 15.46 g/L, while the cellular astaxanthin content increased by 13.33%, demonstrating that yeast extract can promote both the cell growth and accumulation of astaxanthin. Then, using 3% SPJ and adding 0.5 g/L yeast extract as the initial medium, the fermentation was fed with 50% concentrated SPJ to achieve 45.20 g/L biomass for 120 h. It can be easily observed that the cultivation of *P. rhodozyma* using SPJ achieves a higher biomass with shorter time than other inexpensive raw materials of plant origin.

The protein content is about 0.6% of the dry matter in SPJ. In this paper, the pH of SPJ was adjusted to 4.0 with HCl, resulting in a precipitation which has a 1% of dry matter (including 60% of the protein). When *P. rhodozyma* was cultured with SPJ without acid-precipitated protein, higher biomass and cellular astaxanthin were acquired. We further analyzed the precipitation and found that the acid precipitation process not only removed the sweet potato protein but also probably removed the polyphenols [32] in SPJ and other constitutes that are harmful for cell growth. The result is similar to the reports of Stoklosa et al. [11].

SPJ is a byproduct of sweet potato starch processing and is often treated as industrial wastewater [33]. SPJ containing sucrose, glucose, and fructose could account for up to 60% of SS and is rich in vitamins and trace elements, which makes for a more ideal and inexpensive microbiological medium. For example, Fan et al. [19] used SPJ to culture *Bacillus thuringiensis* as biopesticides, and the final cell counts and spore counts achieved in SPJ medium were 72% and 107%, respectively, higher than those in the soybean meal based medium. In this paper, SPJ was used for the first time as a substrate for culturing *P. rhodozyma*, realizing high-density culture of *P. rhodozyma*.

## 5. Conclusions

In this study, we investigated for the first time the utility of SPJ, a waste material derived from the industrial production of sweet potato starch, as a nutrient source for *P. rhodozyma* in the production of astaxanthin. The biomass and astaxanthin yields of the

culture fluids thus obtained were found to be comparable to those obtained using a composite medium. Moreover, supplementation of this deproteinized SPJ with a small quantity of yeast extract was observed to enhance the biomass production and astaxanthin yield of *P. rhodozyma*. Using this optimized system in conjunction batch feeding in a 5 L fermenter, we obtained a maximal biomass of 45.2 g/L, which in most cases, exceeds the values previously reported. The use of SPJ as a medium for culturing astaxanthin-producing *P. rhodozyma* not only reduces the costs of astaxanthin production using this yeast but also effectively solves the problem of wastewater treatment in the sweet potato starch industry.

## Author contributions

- Study conception and design: C Zhang, X Zhao
- Data collection: C Zhang, Q Li, R Li
- Analysis and interpretation of results: C Zhang, J Zhang, M Yao
- Draft manuscript preparation: L Liu, H Xu, X Zhao
- Revision of the results and approved the final version of the manuscript: Y Tian

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## Conflict of interest

The authors report no potential conflicts of interest.

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