Disruption of *Phaffia rhodozyma* cells and preparation of microencapsulated astaxanthin with high water solubility

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Abstract A novel process was developed for encapsulation of astaxanthin from *Phaffia rhodozyma*. The yeast cells were disrupted by glass beads and the high shearing force partially emulsified the astaxanthin in aqueous phase. The enzymolysis method was then adopted to prepare the yeast extract for a full use of the cells. The gelatin and porous starch were used to microencapsulate the emulsified astaxanthin. Under optimized conditions, the recovery of amino nitrogen and solid reached $3.68 \pm 0.32\%$ and $49.22 \pm 2.34\%$, respectively. The microencapsulation conditions were optimized through orthogonal experiment and the encapsulation efficiency, loading astaxanthin, and amino-nitrogen reached 88.56%, 1.55 mg/g, and $1.35 \pm 0.14\%$, respectively. The water solubility of

microcapsules reached $81.5 \pm 0.35\%$. Color and storage stability analysis showed that microencapsulation of astaxanthin possessed higher thermal stability. The results demonstrated that the established process was effective and practical.

Keywords Disruption · Bead milling · Astaxanthin microencapsulation · Porous starch · Gelatin

Introduction

The past three decades have witnessed an enormous interest in the use of microorganisms (microalgae, marine bacteria, basidiomycete yeast and so on) to obtain metabolism products (Cardozo et al., 2007; Mata-Gómez et al., 2014). The psychrotolerant basidiomycetous yeast *Phaffia rhodozyma* can produce astaxanthin and oligosaccharide simultaneously (Bie and Zhu, 2016). Astaxanthin can be used to enhance the immune system of some diseases, such as cancer (Chew et al., 2011), inflammation (Guerin et al., 2003; Park et al., 2008), and diabetes (Leite et al., 2010; Sila et al., 2015) as well as a pigment additive in aquaculture feed.

Astaxanthin (3, 3'-dihydroxy- β , β -carotene-4, 4'-dione), a liposoluble carotenoid from xanthophylls family, is a predominant pigment in *P. rhodozyma* (Liu et al., 2016). The astaxanthin market accounts for 29% of total carotenoids sales with a global market size of \$ 225 million dollars and is estimated to increase to approximately \$253 million by 2018 (Mata-Gómez et al., 2014). Astaxanthin can be synthesized from petrochemicals by chemical process. However, natural sources of this nutraceutical ingredient is stimulated due to a mixture of stereoisomers of the synthetic product, which is proved as a statistical mixture

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(S: meso: R = 1:2:1) of astaxanthin and less bioavailable than natural 3S, 3'S astaxanthin (Liu et al., 2016). Generally, *P. rhodozyma* has a considerable commercial potential as a food source of astaxanthin for poultry and farmed salmonids (Choi et al., 2016). Currently, the commercial production of astaxanthin is prohibitively costly for the aquaculture industry, suggesting that more research efforts should be made to reduce the cost of natural astaxanthin, such as the use of low-cost raw materials (Bhatt et al., 2013), strain improvement on the metabolic engineering and genetic levels of biosynthetic astaxanthin (Bellora et al., 2016; Najafi et al., 2013).

The thick cell wall severely impedes the availability of astaxanthin though it also protects astaxanthin from degradation. Thus, cell wall disruption is a crucial step to improve astaxanthin bioavailability. To date, various methods have been attempted to disrupt the cell wall of *P. rhodozyma* for astaxanthin extraction (Michelon et al., 2012). It is reported that the mixed culture of *Xanthophyllomyce dendrorhous* and *Bacillus circulans* could successfully extract the major astaxanthin (Fang and Wang, 2002). However, this method is time-consuming and not easily used in industrial production. More recently, mechanical disruption through glass beads is shown to be a simple and promising economical procedure (Lee and Fang, 2011). Astaxanthins are insoluble in aqueous solutions, while high shearing force can partially emulsify astaxanthins without the organic solvent extraction process.

Astaxanthin is unstable due to two principal degradation reactions: isomerisation and oxidation (Villalobos-Castillejos et al., 2013). Hence, microencapsulation is essential to maintain the stability of astaxanthins. Microencapsulation efficiency and microcapsule stability are largely dependent on wall material property (Evi et al., 2015; Varankovich et al., 2017). At present, microencapsulated wall materials are commonly selected from modified starch products, polysaccharide, proteins, gum and so on. Among them, the porous starch surface with a porous structure can greatly improve the adsorption capacity as an adsorbent and is widely used in multiple applications, individually or jointly with other gelling agents (Budarin et al., 2006; Gomez-Estaca et al., 2016). Meanwhile, gelatin is a good choice for microencapsulation due to its good properties of film-formation, water-solubility, edibility, and biodegradation (Jafari et al., 2007).

In the present study, a novel process was developed for encapsulation of astaxanthin from *Phaffia rhodozyma*. The yeast cells were disrupted by glass beads and the high shearing force partially emulsified the astaxanthin in aqueous phase. The enzymolysis method was then adopted to prepare the yeast extract for a full use of the cells. The gelatin and porous starch were used to microencapsulate the emulsified astaxanthin. The FT-IR spectra, water

solubility, color and stability of the astaxanthin microcapsules were also analyzed to verify the process.

Materials and methods

Materials

Phaffia rhodozyma Y119 was stored in the Institute of Biological Sciences and Engineering of South China University of Technology, Guangzhou, China. Jerusalem artichoke extract was obtained as previously reported (Jiang et al., 2017). The porous starch was prepared as previously reported (Budarin et al., 2006). Soluble starch was purchased from Shanghai guoyao reagent group (Shanghai, China), Angel Complex Enzyme (CE) was purchased from Angel Yeast Co., Ltd. (Yi Chang, Hu Bei, China).

Cell harvesting and yeast disruption

The yeast was incubated at 220 rpm and 22.5 °C for 72 h in a 250 mL Erlenmeyer flask at constant temperature incubator shaker controller (New Brunswick Scientific Co., Inc, Newark, New Jersey, USA), containing 22.5 mL medium composed of mixed carbon sources: 30 g/L (ca. glucose: total sugar in Jerusalem artichoke extract = 2:1), 4.75 g/L peptone, 0.2 g/L KNO₃, 3.0 g/L KH₂PO₄, 0.5 g/L K₂HPO₄ and 0.5 g/L MgSO₄. The cells were harvested by centrifugation (Eppendorf, Hamburg, Germany) at 11,515 g for 15 min at 4 °C, and the pellets were resuspended in distilled water for the experiments.

The yeast suspensions were placed in 250 mL glass vessels. Cells were disintegrated with glass beads with a diameter of 1 mm in an orbital shaker at 220 rpm to break the cells and release the inner content. In order to optimize the extraction conditions for total astaxanthin, several single factor experiments were performed under yeast concentration (10, 20, 30, and 40 g/L), temperature (22, 30, 40, and 50 °C), pH (5, 8), and glass bead amount (100, 200, 250, and 300 g/L). The extraction yield of astaxanthin represented the disruption yield of yeast.

Preparation of yeast extract

The mixture obtained in the disruption process was carefully pipetted out, divided into aliquots, and supplemented with different concentrations of angel CE (0.5, 1, 2, and 3% w/w, per dry cell weight) for hydrolyzing protein at 40 °C. After a 5 min boiling water bath to inactivate the angel CE, the hydrolysis yield was estimated by measuring the free amino group content in the yeast hydrolyzate using the formaldehyde titration method.

Preparation of microcapsules and expanding experiment

The porous starch was dissolved in hot distilled water at a concentration of 25%. The yeast extract solution containing the emulsified astaxanthin (at a pigment concentration of 93.72 mg/L) was poured into the porous starch solution under stirring to adsorb the astaxanthin for 20 min, followed by addition of the gelatin to form a coarse emulsion under the conditions shown in Table S1: Yeast extract solution, 20, 30, and 40 mL; Mass of gelatin, 0.7, 1.0, and 1.5 g; Mass of porous starch, 0.5, 0.6, and 0.7 g; Encapsulation temperature, 40, 45, and 50 °C; Encapsulation time, 2, 3, and 4 h.

The yeast suspensions (in the concentration range of 20, 50, 100, and 200 g/L) were placed in 250 mL glass vessels. Glass bead (amount 500 g/L) of 1 mm was used to break the cells at 150 rpm for 48 h. Then the yeast extract was prepared and the astaxanthin was microencapsulated under the optimized conditions via orthogonal experiments. E1, E2, E3 and E4 represent the yeast suspension concentrations of 20, 50, 100 and 200 g/L, respectively.

Thermal stability of encapsulated astaxanthin

Aliquots of the yeast extract and encapsulated yeast extract were accurately weighed and placed in a different incubator at 22, 37, 55 and 105 °C in darkness for 15 days.

Extract yield of astaxanthin

The modified hot acid method was used to analyze the total astaxanthin content (Jiang et al., 2017). Briefly, 2 mL of a certain concentration of yeast suspensions was sampled before disruption and processed to measure the total astaxanthin. The absorbance of the final sample solutions was measured at 474 nm using a UV/visible spectrophotometer. Total astaxanthin was calculated using the 1% extinction coefficient = 1600 according to Eq. (1):

$$\text{Total astaxanthin (mg/g of DCW)} = \frac{A_{474} \times D \times V_2}{0.16 \times W} \quad (1)$$

where A is absorbance; D, dilution ratio; V_1 , dilution volume (mL); W, dry weight of cell (g).

The released astaxanthin was extracted using the method as previously described (An et al., 1989). Briefly, approximately 2 mL of the suspension was sampled after cell disruption and extracted with acetone, and then reextracted carotenoids with petroleum ether. The absorbance of the petroleum ether extract was measured at 474 nm using a UV/visible spectrophotometer. The released astaxanthin

was calculated using the 1% extinction coefficient = 2100 by the Eq. (2):

$$\text{Released astaxanthin (mg/g of DCW)} = \frac{A_{474} \times D \times V_2}{0.21 \times W} \quad (2)$$

where A is absorbance; D, dilution ratio; V_2 , dilution volume (mL); W, dry weight of cell (g).

The extraction yields of astaxanthin were calculated by Eq. (3):

$$\begin{aligned} \text{The extraction yield of astaxanthin(\%)} \\ = \frac{\text{Released astaxanthin}}{\text{Total astaxanthin}} \times 100 \end{aligned} \quad (3)$$

Recovery of amino-N

The recovery of amino-N was analyzed by the formaldehyde titration method (Li et al., 2017). The titration end point was determined with an acidimeter.

Encapsulation efficiency

The encapsulation efficiency (EE) was calculated from the total quantity of astaxanthin present in the microcapsules and the quantity present on the surface (Gomez-Estaca et al., 2016). The surface astaxanthin was determined as follows: 500 mg of freeze-dried powder was accurately weighed and washed twice with 3 mL of acetone, followed by mixing the two volumes of acetone, and the surface astaxanthin content was calculated according to Eq. (1).

For determination of the loading astaxanthin (LA) of the microcapsules, 500 mg of freeze-dried powder was accurately weighed and dissolved in 2 mL distilled water, followed by addition of acetone stirring in a vortex mixer, and then reextracted with petroleum ether. The total astaxanthin in the upper petroleum ether phase was determined according to Eq. (2).

EE was calculated according to Eq. (4):

$$\text{EE} = \frac{\text{Loading astaxanthin} - \text{Surface astaxanthin}}{\text{Loading astaxanthin}} \times 100\% \quad (4)$$

Measurement of FT-IR, solubility, color and storage stability of the microcapsules

Fourier transform infrared spectroscopic (FT-IR) spectra of the samples (astaxanthin yeast extract, gelatin, porous starch and encapsulated complexes) were obtained in the range from 400 to 4000 cm^{-1} using a Perkin-Elmer Spectrum One spectrometer without sample preparation. The resolution was 0.01 cm^{-1} .

The solubility of the microencapsulated astaxanthin was determined using a gravimetric method (Comunian et al., 2013).

The color parameters of the microcapsules with a different particle size were determined using a portable colorimeter (Konica Minolta, CM-700D) as previously described (Anarjan et al., 2012). The C_{ab}^* and total color difference (TCD) were calculated according to Eq. (5) and (6), respectively.

$$C_{ab}^* = (a^{*2} + b^{*2})^{1/2} \quad (5)$$

$$TCD = (L^{*2} + a^{*2} + b^{*2})^{1/2} \quad (6)$$

where L^* , indicates black (−) to white (+); a^* , green (−) to red (+); b^* , blue (−) to yellow (+); C_{ab}^* , intensity of color; TCD, total color difference.

To evaluate the thermal stability of the astaxanthin microcapsules, the samples were stored at 22, 37, 55, 105 °C for different periods of time, and an aliquot of each sample was used to measure the retention rate of the astaxanthin with different average particle size. The astaxanthin present in the microcapsules was measured in the same way as described above. The astaxanthin retention rate (ARR, %) was quantified by Eq. (7):

$$\begin{aligned} \text{ARR}(\%) &= \frac{\text{Encapsulated amount of astaxanthin after storage}}{\text{Encapsulated amount of astaxanthin initially prepared}} \times 100 \end{aligned} \quad (7)$$

Statistical analysis

The values were reported as the mean $\pm$ standard deviation (SD), and all experiments were performed in triplicate. Statistical evaluations were performed by *T* test for the differences between the experimental groups. A value of $p < 0.05$ was considered significant. All statistical analyses were performed using the SPSS software (Ver. 17.0, SPSS Inc., Chicago, IL, USA).

Results and discussion

Disruption of *Phaffia rhodozyma* cells

Efficient cell disruption is essential to increase the extraction yield of astaxanthin and the release of intracellular products for bioavailability. Due to a thick cell wall, *P. rhodozyma* is difficult to hydrolyze, and only a large enzyme dosage could lead to cell disruption. Physical methods, such as the ball mill by Bead-Beater, would

increase local temperature and lead to astaxanthin degradation because of the sharp attrition (16). In the present study, the cell walls of the yeast were successfully disrupted by glass bead in Erlenmeyer flasks due to a low speed (150 rpm), avoiding the heat produced by sharp attrition of the cells. The degree of cell rupture increases under the suitable yeast cell concentration due to the increased ball-cell interaction.

With 20 g dry cell/L, 65% extraction yield was achieved with 200 g/L glass beads for 6 h [Fig. 1(A)]. The extraction yield of astaxanthin was directly related to the cell concentration. It was reported that the increased viscosity and film formation on the surfaces of the beads diminished the effectiveness of the shearing force with an increase of the yeast concentration (Ricci-Silva and Vitolo, 2000), which led to the decreases of effective collision frequency and collision speed. The astaxanthin extraction was basically proportional to cell disruption and increased with the extension of disruption time.

Astaxanthin degradation can be ascribed to many factors, such as extraction and storage conditions (temperature, light and oxygen) (Chen et al., 2007). The temperature during disruption process significantly affected astaxanthin degradation. As shown in Fig. 1(B), an increase of temperature from 22 to 50 °C did not affect astaxanthin extraction in a short time, but the astaxanthin concentration dropped rapidly after a few hours at 50 °C. Milling has no significant impact on the stability of astaxanthin (Anderson and Sunderland, 2001). However, the temperature increase resulted from the milling significantly affects the stability of astaxanthin. The loss of astaxanthin through milling depended on the equipment used, the heat produced and operation time. The lab-scale milling equipment used in this experiment can't control the temperature accurately, probably leading to the astaxanthin loss. The scale-up milling equipment usually has good temperature control apparatus, avoiding the astaxanthin loss.

As shown in Fig. 1(C), the degree of cell disruption in bead mills increased with the number of beads due to the increased bead-yeast interaction. The highest extraction yield of astaxanthin (98%) was obtained at 300 g/L. As expected, a higher glass bead loading would result in more frequent collision of the beads with the yeast cells. However, operating at a higher bead loading was noted to lead to facility damage and increased power consumption. The extraction yield can also reach 98% with traditional milling method (Fonseca et al., 2011), but the astaxanthin were emulsified in aqueous phase avoiding the procedure of organic solvent extraction in this study.

Fig. 1 Effects of different factors on the disruption of *Phaffia rhodozyma* cells. (A) Yeast concentration (10, 20, 30, and 40 g/L), temperature (22 °C), bead amount (200 g/L), (B) temperature (22, 30,

40, and 50 °C), yeast concentration (20 g/L), bead amount (200 g/L), and (C) bead amount (100, 200, 250, and 300 g/L), yeast concentration (20 g/L), temperature (40 °C)

Preparation of yeast extract

The total nitrogen corresponds to proteins, peptides, free amino acids and non-protein nitrogen. The yeast crude protein content was determined from total nitrogen using a Kjeldahl N factor conversion of 6.25. The protein content of the raw material (dried yeast cell) was determined as 32.2%. The release of yeast components was determined by measuring the amino-N content and soluble solid content.

In the present study, the yeast extract preparation was treated with two enzymatic hydrolysis processes: adding angel CE after yeast cell disruption and adding glass beads and angel CE simultaneously. The influence of different processes and dosages on the recovery of solid and protein are shown in Fig. 2. The two different processes varied significantly in their results, and the angel CE worked well after breaking the yeast cell wall. After the treatment for 24 h, no significant difference was found in amino-N recovery (within the range 3.51–3.65%) for different dosages of angel CE. However, the recovery of amino-N was significantly lower for simultaneous addition of glass beads and angel CE.

Microencapsulation of astaxanthin

Gelatin coacervation method can reduce the color staining effect and enhance the stability of astaxanthin. To determine the optimal condition of astaxanthin microencapsulation process, orthogonal design was adopted, and the results are shown in Table 1.

The mass of gelatin showed significant effects on EE ($p < 0.05$), while the other factors had relatively minor influence on EE ($p > 0.05$). The LA was more significantly influenced by the factors of M of gelatin, M of porous starch and yeast extract ($p < 0.05$). According to the values of K1, K2, K3, K4, K5 and K6, the high EE (88.56%) and LA (1.55 mg/g) were observed in experiment NO.8 under the optimal conditions: yeast extract, 40 mL; M of gelatin, 1.5 g; M of porous starch, 0.5 g; microencapsulation temperature, 40 °C; microencapsulation time, 2 h (Table 1). The final content of amino-nitrogen of the microencapsulated powder was determined as $1.35 \pm 0.14\%$, indicating that the microencapsulation had no significant effect on the release of amino-nitrogen.

Fig. 2 Effects of enzyme loading on the yeast extract preparation. **A** Recovery of amino-N, and **B** recovery of solid

Table 1 The result of orthogonal design for the optimization of astaxanthin microencapsulation

No.	Yeast extract (mL)	Mass of gelatin (g)	Mass of porous starch (g)	Encapsulation temperature (°C)	Encapsulation time (h)	EE (%)	LA (mg/g)
1	20	1.5	0.7	50	3	80.19	0.61
2	30	1	0.6	40	3	67.32	0.98
3	40	1	0.7	40	4	58.88	1.03
4	20	1.5	0.6	50	4	74.10	0.57
5	40	1	0.5	50	3	53.62	0.94
6	30	1.5	0.7	45	2	81.81	1.19
7	40	0.7	0.7	45	4	54.90	0.96
8	40	1.5	0.6	40	2	88.56	1.55
9	40	0.7	0.6	50	2	51.93	0.91
10	30	0.7	0.6	45	3	62.12	0.90
11	20	0.7	0.7	40	3	73.82	0.56
12	30	1	0.7	50	2	56.59	0.82
13	40	1.5	0.5	45	3	79.19	1.38
14	30	1.5	0.5	40	4	83.82	1.22
15	20	1	0.5	45	2	76.08	0.58
16	20	1	0.6	45	4	77.17	0.59
17	30	0.7	0.5	50	4	48.95	0.71
18	20	0.7	0.5	40	2	51.31	0.39
K ₁	432.65	343.02	392.96	423.71	406.28		
K ₂	400.61	389.65	421.20	418.09	416.25		
K ₃	387.08	487.67	406.18	378.55	397.82		
K ₄	3.31	4.44	5.22	5.73	5.44		
K ₅	5.81	4.94	5.49	5.41	5.38		
K ₆	6.77	6.51	5.17	4.75	5.07		

EE encapsulation efficiency, LA loading astaxanthin

K₁, K₂ and K₃ come from the results of encapsulation efficiency

K₄, K₅ and K₆ come from the results of loading astaxanthin

Expanding experiment

The low LA under the above optimal conditions was most likely attributed to the low concentration of astaxanthin in the yeast extract. Therefore, we increased the concentration of yeast at the wall-breaking stage, extended the disruption time, obtained a different concentration of astaxanthin, and microencapsulated the astaxanthin according to the optimized conditions.

In Table S2, it can be seen that the astaxanthin concentration in aqueous phase was elevated with an increase of the yeast concentration. However, the astaxanthin content decreased due to enhanced viscosity when the yeast concentration reached a certain extent. At the yeast concentration of 100 g/L, the astaxanthin concentration reached 1043.17 mg/L, and the LA was 10.42 mg/g.

Characterization of encapsulated astaxanthin

Figure S1 shows the FT-IR spectra of gelatin, porous starch, astaxanthin, yeast extract and microcapsules powder. The spectra for the microencapsulated powder (Fig.S1 E) were very similar to those of gelatin (Fig.S1 B), which means the formation of microencapsulation (Chen et al., 2007). Characteristic extract bands such as those at 1651 and 1051 cm^{-1} almost disappeared in the spectra of the encapsulated complexes, indicating the occurrence of microencapsulation. The bands at 1077 and 1002 cm^{-1} attributed to C–OH and C–O–C were prominent in the spectra of the porous starch (Fig.S1 A), indicating the porous starch worked as the adsorbent. The occurrence of encapsulation can be determined by the change of absorption peaks, such as displacement or disappearance of the absorption peak, which is usually evidenced by comparing the spectra of gelatin, porous starch, astaxanthin, yeast extract and microcapsules.

Solubility assay of microencapsulated astaxanthin

In the solubility assay, no deposit was observed in the solution, whose color and luster were vivid and transparent. As shown in Fig. 3, the solubility of the encapsulated astaxanthin was further improved, with no significant difference ($p > 0.05$) among particle sizes over 40 mesh despite obvious color differences for different size microcapsules. All samples were readily soluble in water without any macroscopic precipitation. The solubility of microencapsulated astaxanthin (73.12–81.5%) greatly exceed microencapsulation of astaxanthin with blends of milk protein and fiber (Chen et al., 2007; Shen and Quek, 2014). The undissolvable characteristics of astaxanthin will limit its potential application in foods, especially beverages. The solubility data obtained in this study expanded the

Fig. 3 Solubility indexes of microencapsulated powder with a different particle size (PS). ^{a, b} different letters show statistically significant ($p < 0.05$) differences

applicability of astaxanthin in a wide variety of foods such as jellies, candy desserts, soft drinks, popsicles, and others.

Color measurement of microencapsulated astaxanthin

The appearance and color of the microencapsulated powder were important visual factors that contribute to customer selection. The color of the encapsulated astaxanthin depended on the wall material and the particle size distribution (Anarjan et al., 2012). The color parameters of as-prepared astaxanthin particles were shown in Table 2.

The microencapsulated particles prepared under optimized conditions had different particle sizes. A comparison of the particle size of the encapsulated astaxanthin and the color parameters revealed that the smaller particles became redder, yellower and lighter. The smaller the particles were, the larger the surface area would be, leading to an increase in absorption coefficient and scattering coefficient of microcapsule particles. A previous study has reported that the higher a^* , b^* , and C_{ab}^* values for microencapsulation signified the higher chemical stability (Comunian et al., 2013). However, in this study, for the same astaxanthin microcapsules with a different particle size, the small particle size possessed higher color parameter values, but a lower thermal stability value (Fig. 4).

Storage stability of microencapsulated astaxanthin

Thermal degradation of astaxanthin was a complex process with two principal reactions: isomerisation and oxidation. The stability of astaxanthin microcapsules strongly depended on the wall materials and the environmental

Table 2 Color parameters of microencapsulated powder with a different particles size

Particle size	L*	a*	b*	C _{ab} *	TCD
PS > 40	38.87 ± 0.06 ^a	21.79 ± 0.34 ^a	10.83 ± 0.68 ^a	24.33	45.86
100 < PS ≤ 40	40.15 ± 0.23 ^b	27.05 ± 0.13 ^b	14.22 ± 0.05 ^b	30.56	50.46
200 < PS ≤ 100	47.78 ± 0.53 ^c	28.19 ± 0.45 ^c	17.4 ± 0.17 ^c	33.13	58.14
PS ≤ 200	50.82 ± 1.58 ^d	30.58 ± 0.12 ^d	23.7 ± 0.37 ^d	38.69	63.87

^{a-d}Different letters show statistically significant ($p < 0.05$) differences between response values of each column

L*, black (−) to white (+); a*, green (−) to red (+); b*, blue (−) to yellow (+); C_{ab}*, intensity of color; TCD, total color difference

Fig. 4 The effect of temperature on astaxanthin retention. **A** 22 °C, **B** 37 °C, **C** 55 °C, and **D** 105 °C

conditions. The storage stability of different particle size microcapsules was assessed by the astaxanthin retention at different temperatures (22, 37, 55, and 105 °C) for various periods of time. As shown in Fig. 4, the temperature increase accelerated the degradation of the astaxanthin microcapsules.

During the 10 days of storage [Fig. 4(D)], the ARR with a different particle size showed a significant decrease, but the decrease was greater for the small size astaxanthin

microcapsules (PS ≤ 200 mesh) than the larger size (PS > 40 mesh). The thermal stability of astaxanthin microcapsules increased with the particle size. The same phenomenon occurred at 22, 37, and 55 °C [Fig. 4(A–C)]. These results implied that the astaxanthin degradation rates did not follow first-order kinetics under those conditions. Astaxanthin decreased rapidly at the first day at 105 °C, and then leveled off slowly along with increasing heating time. The free astaxanthin on the surface contacted oxygen

first and was easy to be degraded. The smaller the particle is, the greater the specific surface area will be, which leads to a reduction in ARR. Overall, oxidation reaction played a dominant role in the thermal degradation of astaxanthin, and the macroaggregate (≥ 40 mesh) of astaxanthin microcapsules contributed to storage stability.

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Compliance with ethical standards

Conflict of interest The author declares that they have no conflict of interest.

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