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Full Length Research Paper

Different cell disruption methods for astaxanthin recovery by *Phaffia rhodozyma*

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Astaxanthin (3,3'-dihydroxy-b,b'-carotene-4,4'-dione) is carotenoid of high market value whose demand has increased in such fields as aquaculture, pharmaceutical supplements and natural coloring. Cell disruption is the first step for isolating intracellular materials and it depends on the cell wall permeability. In order to maximize the recovery of astaxanthin from *Phaffia rhodozyma* NRRL-Y17268, drying and freeze pretreatments were tested by different cell disruption methods: abrasion with celite, glass pearls in vortex agitator, ultrasonic waves, sodium carbonate (Na_2CO_3) and dimethyl sulfoxide (DMSO). The method with Na_2CO_3 was not effective; meanwhile, the agitator with glass pearls, the abrasion with celite and the ultrasonic waves were found as promising for future studies. As a result, the DMSO in freeze-dried biomass with 4 process cycles and biomass/DMSO relation of 0.025 g/ml was found to be the most efficient for analytical determination, increasing about up to 25 times the astaxanthin recovery.

Key words: Carotenoids, yeast, chemical disruption, dimethyl sulfoxide.

INTRODUCTION

Astaxanthin (3,3'-dihydroxy-b,b'-carotene-4,4'-dione) is an oxygenated carotenoid that has a high market value and an increasing demand. Interest in such a pigment is found in various fields as aquaculture, pharmaceutical supplements and natural coloring (Liu and Wu, 2006; Moriel et al., 2005; Dutta et al., 2005).

According to Liu and Wu (2006) and (Moriel et al. (2005)), the pink color of the salmon and of the peels of cooked crustaceans is due to the astaxanthin presence, which also contributes to the aroma of these meats after cooking. In aquaculture, rearing salmonids and crustaceans in pen culture systems requires a diet containing astaxanthin to obtain the appropriate coloration (Nakano and Takeuchi, 1995), as they cannot synthesize carotenoids (Liu and Wu, 2006; Moriel et al., 2005). Besides pigmenting the fish muscle, astaxanthin improved the health of fishes, increasing the performance of liver and tissues, possibly inhibiting the generation of reactive oxygen and preventing lipoproteins from oxidizing. Astaxanthin has various fundamental biological functions for these fishes, such as: protection against oxidation of polyunsaturated fatty acids; protection from the effects of UV rays; immune answer; reproductive behavior and improving reproduction (Guerin et al., 2003). In domestic bird rearing, astaxanthin is used for increasing the coloration of egg yolk, resulting in a better quality and acceptance in the consumer market (Liu and Wu, 2006; Moriel et al., 2005).

Carotenoids have considerable nutritional benefits due to its antioxidant effect and the potential to impede or delay degenerative diseases such as arteriosclerosis, cancer and aging; as well as it increases the immune answers in animals and humans. The antioxidant properties of astaxanthin play an important role in protection against photo-oxidation from light UV, inflammations, cancer, infectious ulcers from *Helicobacter pylorii*, aging and diseases related to age or in health promotion,

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Abbreviation: DMSO, Dimethyl sulfoxide.

immune answer and performance of liver, heart, eyes, articulations and prostate (O'Connor and O'Brien, 1998).

The pigments produced by biological methods have emerged as a growing segment of the industrial market. Besides being a nutritional supplement, they are also used in cosmetic and pharmaceutical industries as well as in aquaculture. Process development and use of new and available technologies for production optimization are important research fields on natural food grade colors. Because of the increasing worldwide market, the high cost of synthetic astaxanthin and the need to obtain astaxanthin from natural sources at low costs and high productivity. The yeast *Phaffia rhodozyma* has been studied for producing this carotenoid by submerged culture (Chattopadhyay et al., 2008; Liu and Wu, 2006; Moriel et al., 2005).

The cell disruption is the first step in the isolation of intracellular materials, being a key step in the downstream process, it has considerable influence not only on the total amount of material to be recovered, but also on its biological activity, its association with other cell components and the possible presence of proteolytic degradation and contaminants which may affect the subsequent purification steps. Cell disruption is the key for producing and purifying intracellular substances and it has important effects on the extract recovery and quality (Becerra et al., 2001; Buxadó et al., 2004), as well as it is of extreme importance for analytical aims.

Because of its larger size and distinct cell wall structure, disruption of yeasts is generally easier than the one of bacteria. The basic components of the cell wall of yeasts are glucans, manans and proteins. Usually, mechanical methods are not specific but they still have higher efficiency and wider application than other methods. Yet chemical disruption is a possible substitute for the mechanical one due to its simplicity, relative speed and low cost (Choe and Middelberg, 2001).

Furthermore, characteristics of disruption process may vary according to the mechanical stability of the microorganism, which depends on species, culture time, specific growth rate, culture temperature and culture medium (Becerra et al., 2001; Geciova et al., 2002). Different methods of disruption can be used for extracting intracellular substance. They depend on the physical force of the cell wall, location inside the cell, stability and the use wanted for the compound (Kula, 1990; Liu and Wu, 2006). Mechanical, physical, chemical and enzymatic methods, as well as the combination of these can be used (Geciova et al., 2002). Such methods of cell wall disruption have been studied for yeasts by using ultrasonic waves associated with glass pearls to extract β-galactosidase enzyme by *Kluyveromyces marxianus* CCT 7081 (Medeiros et al., 2008). Different solvents (DMSO, petroleum ether, hexane, ethyl acetate, chloroform and acetone), liquid N_2 and diatomaceous earth were used to disrupt the cell of *Salmonicolor Sporidiobolus* CBS 2636, thus, recovering the intracellular carotenoids (Valduga et al, 2009).

An improved process for cell disruption and astaxanthin extraction from *P. rhodozyma* was studied using an autoclave at low acid concentration under the optimum conditions (HCl 0.5 M and autoclave pressure 0.1 Mpa, 15 min) and the scanning electron microscopy pictures showed that, all yeast cells shattered into fragments after autoclave treatment at low acid concentration condition, whereas, cells were intact or partly broken after treatment by some other physical and chemical processes. This new method left no residual toxin and gave higher extraction recovery, with good prospects for industrial use (Xiao et al., 2009).

This study aimed to evaluate the drying and freeze pretreatments of biomass by different cell disruption methods: abrasion with celite, glass pearls in vortex agitator, ultrasonic waves, bicarbonate of sodium and dimethyl sulfoxide, in order to maximize astaxanthin recovery and preserving properties by *P. rhodozyma* NRRL-Y 17268 and to compare such disruption processes for analytical purposes.

MATERIALS AND METHODS

Microorganism

The yeast *P. rhodozyma* NRRL-Y 17268 was kept at 4°C in inclined agar yeast malt (YM), composed by (g/l) extract of yeast (3.0), extract of malt (3.0), peptone (5.0), glucose (10.0) and agar (20.0) and stored to be used in the inoculum.

Inoculum

The yeast from the culture in inclined Yeast Mold (YM) agar was suspended in 2 ml of peptone water 0.1%, added with 10 ml of the YM broth and kept at 25°C for 48 h. For inoculum, Erlenmeyer of 500 ml were prepared with 100 ml of the medium consisting of (g/l) 3.0 yeast extract, 3.0 malt extract, 5.0 peptone, 10.0 glucose and 0.2 KNO_3 (Parajó et al., 1997; Parajó et al., 1998). Then, they were added with 10 ml of suspension and kept at 25°C for 48 h and 150 rpm.

Culture conditions

The culture was incubated in orbital shaker at 25°C and 150 rpm for 168 h, initiated by the addition of 10% of inoculum. The biomass with astaxanthin used for the disruption assays, was obtained by submerged culture using the same medium of inoculum (Parajó et al., 1997; Valduga et al., 2009).

Biomass determination

Biomass was determined by optical density using a spectrophotometer at 620 nm, and converted into dried cell mass by the conversion factor of the standard curve of dry biomass (Kusdiyantini et al., 1998).

Biomass pretreatments

The medium fermented was centrifuged 1745 x g for 15 min, after

Table 1. Pretreatments of *P.rhodozyma* biomass to evaluate different methods of disruption.

Assay	Pretreatment 1 (drying*)	Pretreatment 2 (freeze**)
1	Absence	Absence
2	Absence	Presence
3	Presence	Absence
4	Presence	Presence

* Period, 48 h; Temperature, 35ºC.
** Period, 48 h; Temperature, -18ºC.

the cells were washed with distilled water and centrifuged again in the same conditions, being discarded the supernatant. The precipitate biomass was submitted for pretreatments described in Table 1, for subsequent cellular disruption evaluating the physical methods, mechanics and chemical.

Assays of cell disruption

Assays of cell disruption were carried out in triplicate at least, after the biomass pretreatments resulting in the astaxanthin specific concentration. Next was disruption cell in the mechanical methods, the biomass was then centrifuged at 1745 x g for 10 min (Persike et al., 2002) and the astaxanthin specific concentration was determined in the supernatant.

Mechanical methods

Abrasion with celite

In mortar and pistil, 0.5 g of cells was triturated with 0.5 g of celite, to which 6 ml of acetone were added in order to extract the carotenoid (Valduga et al., 2007).

Abrasion with glass pearls in vortex agitator

Flasks containing 0.5 g of biomass were put in vortex agitator with 0.2 g of glass pearls of 0.25 mm diameter and 6 ml of acetone and agitated for 10 min.

Ultrasonic waves

Amber flasks containing 0.5 g of biomass and 6 ml of acetone were taken in the sonicator bath (power of 40 W RMS and frequency of 20 kHz) with cold water level at half of the recommended limit and 4 cycles of 10 min each were performed (Medeiros et al., 2008).

Chemical methods

Sodium bicarbonate

In Erlenmeyer flasks of 500 ml, 18 ml of Na_2CO_3 0.1 M was added with 6 g of biomass until a homogeneous solution. The container was closed with a cotton wad and bathed at 40 to 45°C for 24 h. Next, centrifugation was performed at 290 x g for 15 min for the cell debris to silt up and the astaxanthin specific concentration was determined in the supernatant. The supernatant volume was measured and frozen (Sabaj, 1979) for determining the astaxanthin specific concentration.

Dimethyl sulfoxide (DMSO)

Centrifuge tubes were filled with 2 ml of DMSO, pre-warmed at 55°C for 30 min in 0.5 g of biomass and strong shaking was carried out in vortex agitator for 1 min and then, left for 30 min at rest. Next, it was added 6 ml of acetone and the solution was homogenized and centrifuged at 1745 x g for 10 min and the supernatant was collected (Persike et al., 2002). The same procedure was repeated for the precipitate. Supernatants were gathered for determining the astaxanthin specific concentration (Sedmak et al., 1990).

Extraction and determination of astaxanthin

In the supernatant obtained were added 10 ml of NaCl 200 g/l and 10 ml of petroleum ether. After the agitation, the solvent phase was separated. To ensure extraction, 5 ml of petroleum ether were added. The solvent phase was filtrated with Na_2SO_4 to eliminate the moisture. Absorbance was measured at 474 nm and the astaxanthin specific concentration was calculated by Equation 1 (Moriel et al., 2004; Moriel et al., 2005).

$$C = (A * V * 10^6)/(A_{1cm}^{1\%} * 100 * m) \quad (1)$$

Statistical analysis

All the assays of cell disruption were carried out in triplicate and treated by analysis of variance (ANOVA), followed by Tukey's test, using the software Statistica 6.0 (Statsoft, Tulsa, OK, USA). The results (averages ± standard deviations) followed by equal letters indicate that, no significant difference was found (Tukey's test). All analyses were performed considering a confidence level of 95% ($p < 0.05$).

RESULTS AND DISCUSSION

Cell disruption by chemical method with Na_2CO_3 was not effective in any of the pretreatments under study, presumably due to the cell wall structure and composition of the yeast *P. rhodozyma*, which is mainly composed of glucan and responsible for the cell rigidity before mechanical shocks and osmotic disequilibria (Fleuri and Sato, 2005). Although, Na_2CO_3 is a classic method for the cell

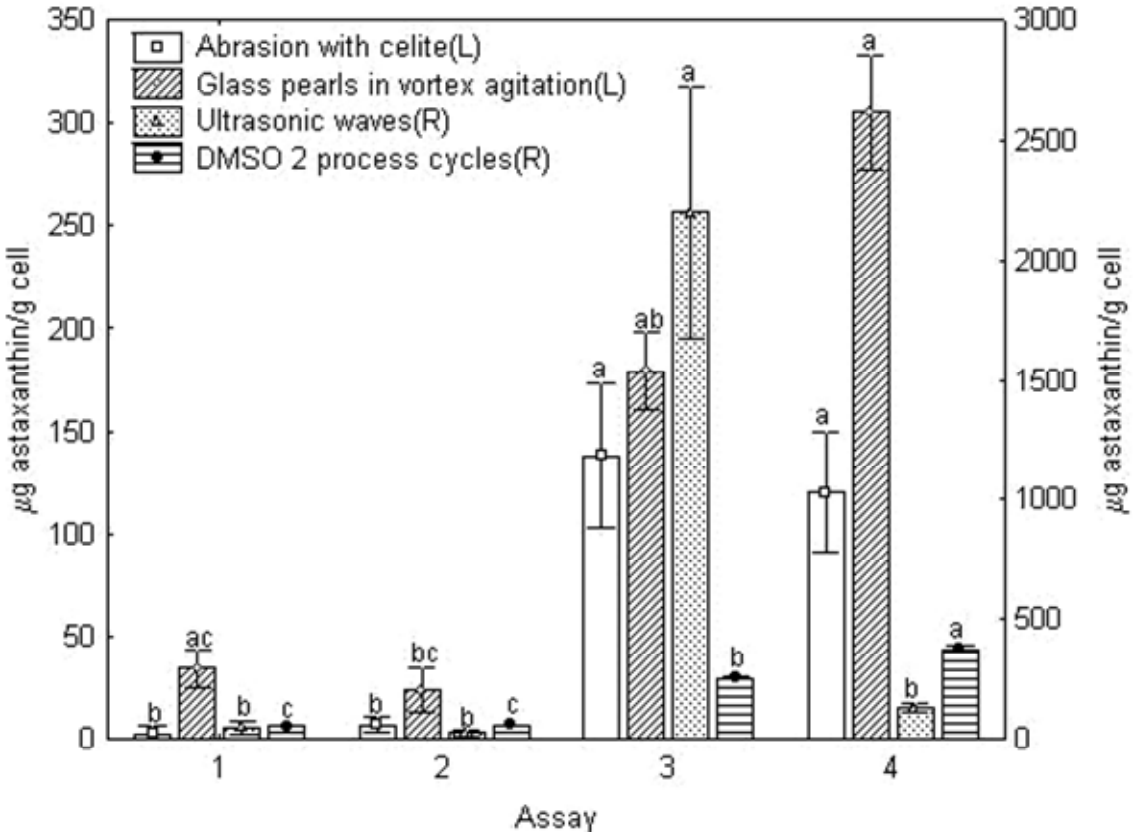

Figure 1. Astaxanthin specific concentration ± standard deviation for disruption method by abrasion with celite, glass pearls in vortex agitator, ultrasonic waves and DMSO in 2 process cycles. Assay 1, (fresh biomass); assay 2, (frozen biomass); assay 3, (dry biomass); assay 4 (dry and frozen biomass). L, left-axes; R, right-axes. The coincidence of one or more letters in the same method of cell disruption indicates that there is no difference at the significance level of 5% (Tukey test).

wall disruption of the yeast *Saccharomyces cerevisae* at concentration of at least 30 mM, it is obtained by extraction of protein covalently linked to some carbohydrates of the cell wall (Fleuri and Sato, 2005), which has glucan in much lesser amount than in *P. rhodozyma*.

Results for the astaxanthin specific concentration from cell disruption by abrasion with celite are shown in Figure 1. Tests of differences among the averages was able to verify that the cell disruption was most effective on the drying pretreatment (assay 3), as evidenced by the significant increase in the astaxanthin specific concentration (138.1 ± 35.0 µg/g). Recovery of such carotenoid through cell disruption by abrasion was not influenced by the freeze pretreatment, since the astaxanthin concentration with fresh (assay 1) and frozen (assay 2) biomass were statistically the same, with a similar occurrence effect when compared with assays 3 and 4. However, when comparing the assays 1 and 3 and 2 and 4, between them is the drying pretreatment found as effective for the cell wall disruption.

The astaxanthin specific concentration from cell disruption by vortex agitator with glass pearls is shown in Figure 1. Results were found similar to the ones of disruption by celite. It may be explained because both methods have the disruption principle based on the physical abrasion of cells with their respective agents. The maximum astaxanthin specific concentration obtained for the disruption using the drying and freeze pretreatments was 305.3 ± 27.7 µg/g. Such behavior may have occurred as the drying pretreatment decreased the water outside the cell, thus, increasing the surface contact with the abrasive and favoring the disruption. Also, since carotenoids are liposolubles, water absence facilitates its extraction.

As shown in Figure 1, the use of ultrasonic waves for cell disruption was efficient for the drying pretreatment only (assay 3), which reached an astaxanthin specific concentration of 2198.4 ± 523.7 µg/g. However, Tukey's test was expected not to show statistical difference between the assays 3 and 4, where the biomass was submitted to drying and freeze pretreatments, resulting in 128.5 ± 19.2 µg/g of astaxanthin. The mechanism of cell disruption is associated with the cavitation phenomena, a shear stress developed by viscous dissipative eddies

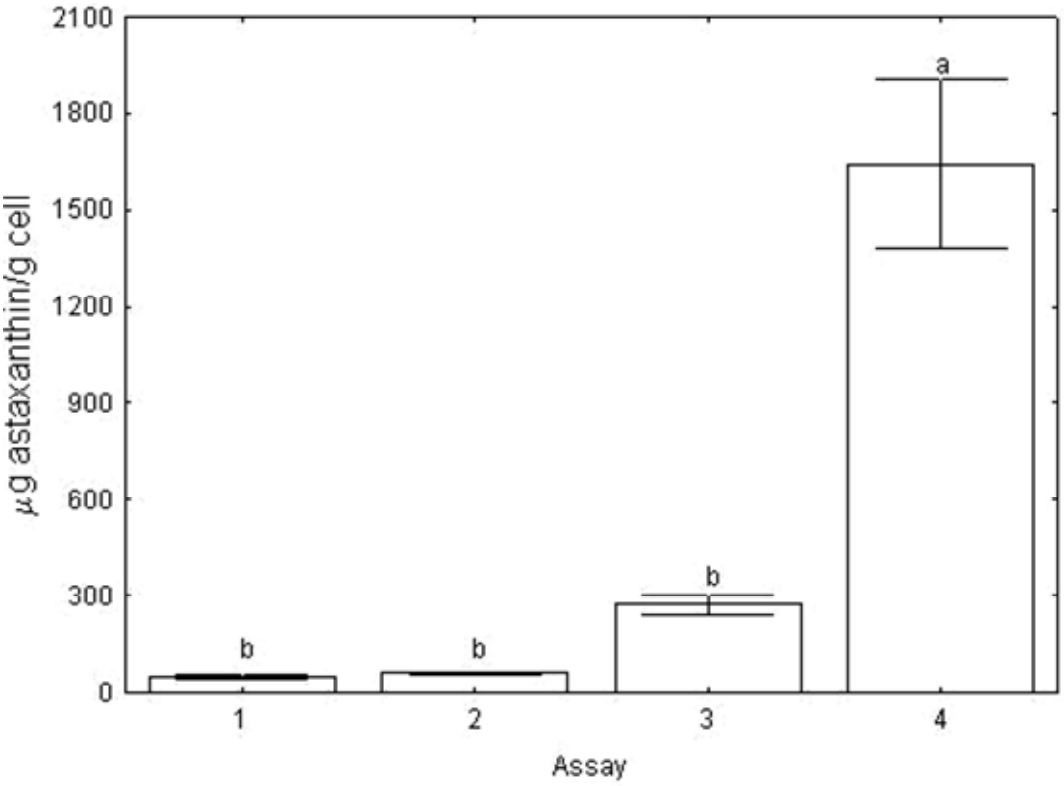

Figure 2. Astaxanthin specific concentration ± standard deviation for disruption method by DMSO in 2-4 process cycles. Assay 1, (fresh biomass); assay 2, (frozen biomass); assay 3, (dry biomass); assay 4, (dry and frozen biomass). The coincidence of one or more letters in the columns indicates that there is no difference at the significance level of 5% (Tukey test).

arising from shock waves produced by imploding cavitation bubbles (Geciova et al., 2002). Presumably, disruption in assay 4 was not effective because the cells were smaller than the eddies formed by ultrasonic waves, generating inactivation of cell growth only.

As far as the process herein referred to was of simple execution, further studies regarding sonication are yet to be developed, such as increasing equipment power or reducing suspension volume, being useful for instance in analytical determination. However, its use in large scale is not recommended, whereas, in terms of eddies, increasing the sample amount means reducing the power dispersed per unit volume, resulting in decreased disruption efficiency. Therefore, constant rate increases linearly with the power and decreases linearly with the working volume. As most of the ultrasonic energy is absorbed into the cell suspensions, it is often overheated, and effective temperature control is necessary so that it does not degrade carotenoid (Geciova et al., 2002).

Results for the astaxanthin specific concentration by chemical agent DMSO are shown in Figures 1 and 2, respectively with 2 and 2 to 4 cycles of cell disruption. As shown in Figure 1, the maximum astaxanthin recovery, statistically superior to the other assays, was found in the cell drying and freeze pretreatments with 375.7 ± 10.5 µg/g (assay 4), which proved the hypothesis that the amount of water outside the cell, as well as the free water, influences on the processes of cell disruption and extraction of carotenoids. Thus, in chemical method of disruption with DMSO, the freeze pretreatment developed the effectiveness of astaxanthin recovery probably because the reduced temperature increased sensitivity. Furthermore, it caused damages to cell membrane due to the ice both outside and inside the cells, the concentration of solutes and the cell dehydration by the increased osmotic pressure (Mazur, 1970).

More cycles of disruption were used (Figure 2), the pretreatments of freeze (assay 2), drying (assay 3) and the fresh biomass (assay 1) had been necessary 3 cycles of disruption. However, freeze-dried cells have more difficulty in being involved, because they are in the form of flakes higher. Thus, the DMSO has its contact reduced with the biomass, which was necessary 4 cycles to achieve total disruption, reaching an astaxanthin specific concentration of 1645.3 ± 263.8 µg/g.

As shown in Figure 3, considering that the maximum astaxanthin recovery was obtained by the drying and freeze pretreatments with 4 cycles of disruption by the chemical method with DMSO, the influence of the biomass/DMSO relation (0.005 - 0.25 g/ml) on the asta-

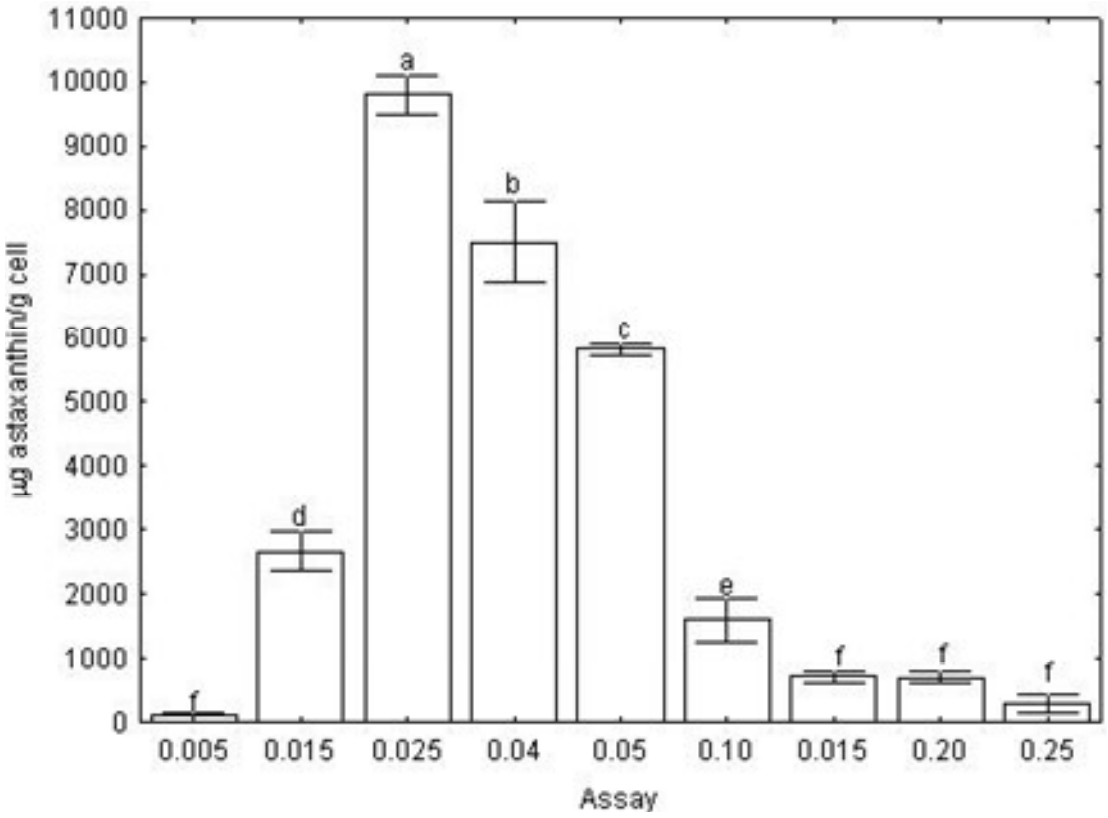

Figure 3. Astaxanthin specific concentration ± standard deviation regarding biomass/solvent relation for disruption method with DMSO in n process cycles by drying and freeze pretreatments. The coincidence of one or more letters in the columns indicates that there is no difference at the significance level of 5% (Tukey test).

xanthin recovery was evaluated under such conditions.

In Figure 3, the maximum astaxanthin recovery was reached for the biomass/DMSO relation of 0.025 g/ml, obtaining 9806.3 ± 292.3 µg/g of astaxanthin in the cell, which differs statistically from the other relations. It demonstrated that, the maximization of intracellular astaxanthin recovery method increased the carotenoid extraction 25 times in comparison with the cell disruption using DMSO, having it dried and frozen with 2 cycles of process and biomass/disruption agent relation of 0.25 g/ml (assay 4 of Figure 1).

Therefore, results show that the increase of disruption process cycles, the biomass amount reduction or the DMSO amount increase represents an improvement in the method in search of higher reproducibility, decreasing the standard deviations of the analysis, with respect to the mechanical methods of cell disruption under analysis, although, they reached cell membrane disruption, they had a high standard deviation in relation to the average. Nevertheless, mechanical process is promising for future studies on reproducibility, since the downstream process used has not proven effective in the removal of abrasive agents from the solution, thereby hindering the recovery of astaxanthin which result in losses that ranged from the stated results. So, cell disruption using DMSO was chosen for analytical aims.

The maximum astaxanthin specific concentration obtained in this study was 9806.3 ± 292.3 µg/g, being superior to the 303.3 µg/g reached by Moriel et al. (2004) for *P. rhodozyma* ATCC 24202 through the method of disruption, extraction and quantification as recommended by Bonfim (1999). Valduga et al. (2009) reached total carotenoids specific concentration of 253.8 ± 485 µg/g, demonstrating the synergistic effect in the disruption of the cell wall of *Salmonicolor Sporidiobolus* 2636 CBS, recovery of pigments with the combination of N_2 líquido/ DMSO and extraction with solvent acetone/methanol (7:3 v/v).

Conclusion

The method of cell disruption with Na_2CO_3 was not effective. In the studied conditions, the disruptions for abrasive methods with vortex agitator together with glass pearls and the celite are promising only for future studies of large scale processes. Moreover, the use of ultrasonic waves for 10 min was efficient for disruption of the cell wall of the yeast *P. rhodozyma* and can be improved in order to use it as alternative to other methods for analy-

tical determination.

The best astaxanthin results were found from the DMSO used as disruption agent in freeze-dried biomass with 4 process cycles and biomass/DMSO relation of 0.025 g/ml, with an optimization of about up to 25 times in astaxanthin recovery, being more efficient for analytical determination than the other methods under study.

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Symbols: C, Astaxanthin specific concentration (µg/g); rpm, rotations per minute; a, absorbance; v, volume (ml); m, cell mass (g); $A^{1\%}_{1cm}$, specific absorption coefficient of 1 g/100 ml, being used the specific absorptivity for xanthophyll, which is 1600 (Davies, 1976).

REFERENCES

Becerra M, Belmonte ER, Cerdán ME, Siso MIG (2001). Extraction of intra cell proteins from *Kluyveromyces lactis*. Food Technol. Biotechnol. 39: 135-139.

Buxadó JA, Heynngnezz LE, Juiz AG, Tamayo G, Lima IR, Marshalleck HD, Mola EL (2004). Scale-up of processes to isolate the misstargeted rBm86 protein from *Pichia pastoris.* Afr. J. Biotechnol. 11: 599-605.

Bonfim TMB (1999). Produção de Astaxantina pela Levedura Phaffia rhodozyma (Xanthophyllomyces dendrorhous) a Partir de Meios de Cultura de Baixo Custo. PhD thesis, Universidade Federal do Paraná, Curitiba, Brazil.

Chattopadhyay P, Chatterjee S, Sen SK (2008). Biotechnological potential of natural food grade biocolorants. Afr. J. Biotechnol. 17: 2972-2985.

Dutta D, Chaudhuri UR, Chakraborty R (2005). Structure, health benefits, antioxidant property and processing and storage of carotenoids. Afr. J. Biotechnol. 13: 1510-1520.

Choe WS, Middelberg APJ (2001). Direct chemical extraction of a recombinant viral coat protein from *Escherichia coli* at high cell density. Biotechnol. Bioeng. 75: 451-455. Doi:10.1002/bit.10064.

Davies BH (1976). Chemical Biochemistry Plant Pigments. Academic press, New York, USA.

Fleuri LFE, Sato HH (2005). Production, purification, cloning and application of lytic enzymes. Quim. Nova 28: 871-879.

Geciova J, Dean B, Jelen P (2002). Methods for disruption of microbial cells for potential use in the dairy industry-a review. Int. Dairy J. 12: 541-553.

Guerin M, Huntley MH, Olaizola M (2003). *Haematococcus* astaxanthin: applications for human health and nutrition. Trends Biotechnol. 21: 210-216.

Kula MR (1990). Trends and future prospects of aqueous two-phase extraction. Bioseparation, 1: 181-189.

Kusdiyantini E, Gaudin P, Goma G, Blanc PJ (1998). Growth kinetics and astaxanthin production of *Phaffia rhodozyma* on glycerol as a carbon source during batch fermentation. Biotechnol. Lett. 20: 929-934.

Liu YS, Wu JY, Ho KP (2006). Characterization of oxygen transfer conditions and their effects on *Phaffia rhodozyma* growth and carotenoid production in shake-flask cultures. Biochem. Eng. J. 27: 331-335.

Mazur P (1970). Cryobiology: The Freezing of Biological Systems. Sci. 168: 939-949.

Medeiros FO, Alves FG, Lisboa CR, Martins DS, Burkert CAV, Kalil SJ (2008). Ultrasonic waves and glass pearls: a new method of extraction of β-galactosidase for use in laboratory. Quim. Nova, 31: 336-339.

Moriel DG, Chociai MB, Machado IMP, Fontana JD, Bonfim TMB (2005). Effect of feeding methods on the astaxanthin production by *Phaffia rhodozyma* in fed-batch Process. Braz. Arch. Biol. Technol. 48: 397-401.

Moriel DG, Machado IMP, Fontana JD, Bonfim TMB (2004). Optimization of biomass and astaxanthin production by the yeast *Phaffia rodozyma*. Braz. J. Pharm. Sci. 40: 421-424.

Nakano T, Tosa ME, Takeuchi MJ (1995). Improvement of bichemical features in fish healt by red yeast and synthetic astaxanthin. Agric. Food Chem. 43: 1570-1573.

O'Connor I, O'Brien NJ (1998). Modulation of UVA light-induced oxidative stress by β-carotene, lutein and astaxanthin in cultured fibroblasts. Dermatol. Sci. 1: 226-230.

Parajó JC, Santos V, Vásques M (1998). Optimization of carotenoid production by *Phaffia rhodozyma* cells grown on xylose. Process Biochem. 33: 181-187.

Parajó JC, Santos V, Vásques M, Cruz JM (1997). Production of carotenoids by *Xanthophyllomyces dendrorhous* growing on enzymatic hydrolysates of prehydrolysed wood. Food Chem. 60: 347-355.

Persike DS, Bonfim TMB, Santos MHR, Lyng SMO, Chiarello MD, Fontana JD (2002). Invertase and urease acivities in the carotenogenic yeast *Xanthophyllomyces dendrorhous.* Bioresour. Technol. 82: 79-85.

Sabaj JAL (1979). Caderno de Práticas de Bioquímica Fundamental. Edgrafurg, Rio Grande, Rio Grande do Sul, Brasil.

Sedmak JJ, Weerasinghe DKE, Jolly SO (1990). Extraction and quantitation of astaxanthin from *Phaffia rhodozyma*. Biotechnol. Tech. 4: 107-112.

Valduga E, Valério A, Tatsch PO, Treichel H, Furigo A, Di Luccio M (2009). Assessment of cell disruption and carotenoids extraction from *Sporidiobolus salmonicolor* (CBS 2636). Food Bioprocess. Technol. 2: 234-238.

Valduga E, Valério A, Treichel H, Di Luccio M (2007). Pretreatment of sugarcane molasses and corn steep liquor for the production of carotenoids. Quim. Nova 30: 1860-1866.

Xiao A, Ni H, Cai H, Li L, Su W, Yang Q (2009). An improved process for cell disruption and astaxanthin extraction from *Phaffia rodozyma*. World J. Microb. Biot. 25: 2029-2034.