INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-6, Issue-1, Jan-Mar-2015 Coden IJABFP-USA

Rceived: 20th Nov-2014 *Revised: 29th Nov-2014* *Accepted: 30th Nov-2014*

Research article

A COMPARISON OF CELL DISRUPTION PROCEDURES FOR THE RECOVERY OF INTRACELLULAR CAROTENOIDS FROM *SPOROBOLOMYCES RUBERRIMUS* H110

Mariana Gomes Clemente Pennacchi[1], Daniel Ernesto Rodríguez-Fernández[2], Francielo Vendruscolo[3], Leila Teresinha Maranho[1], Ivan Marc[4], Lígia Alves da Costa Cardoso[1*]

[1]Positivo University, Professional Master in Industrial Biotechnology, Curitiba, CEP 81.280-330 Paraná, Brazil.

[2]Faculty of Pharmacy, Federal University of Rio de Janeiro, Av. Carlos Chagas Filho 373 CCS, Ilha do Fundão, CEP 21941-902, Rio de Janeiro, Brazil.

[3]School of Agronomy and Food Engineering, Federal University of Goiás-Goiânia, Goiás, Brazil. CEP 74.690-900.

[4]LRGP, UPR CNRS 3349, Lorraine University, Plateforme SVS, 13 rue du bois de la Champelle, 54500, Vandoeuvre-lès-Nancy, France.

[*]Corresponding Author: Phone: +55 41 3317-3449; Fax: +55 41 3317-308;
E-mail: laccardoso@yahoo.fr

ABSTRACT: Carotenoids are intracellular pigments produced by microorganisms, including some species of yeasts, in the stationary phase of growth by the secondary metabolic pathways. In the present study, different methods of *Sporobolomyces ruberrimus* H110 yeast cell lysis were evaluated with the objective of optimizing the recovery of intracellular pigments. Three extraction methods were used: vortex (glass beads and quartz stones), planetary mill (glass beads and quartz stones) and dimethyl sulfoxide. For each one of the lysis agents studied, factorial designs were developed considering as independent variables the agitation speed and lysis agent concentration. A central composite planning was defined considering as independent variables the lysis agent concentration and agitation speed, analyzing as response the estimated total number of extracted carotenoids. From the methods studied, a better extraction of total carotenoid (1.74 $mg.g^{-1}$ of cells and of 1.57 $mg.g^{-1}$ of cells) using the planetary mill method with 135 mg of glass beads or irregular quartz stones, with an agitation speed of 200 rpm. As to the cell lysis, the analysis indicated that the mechanical methods studied showed to be efficient in regards to cell laceration.

Keywords: Carotenoids; Yeast; Cell lysis; Extraction; Planetary mill.

INTRODUCTION

Carotenoids are natural pigments synthesized by most plants and by various microorganisms (Park et al., 2007; Reyes et al., 2014). They are intracellular lipophilic compounds of economic interest, mainly since they act as antioxidant agents and some of them, as precursors to vitamin A (Park et al., 2007; Iturriaga et al., 2000; Davoli and Weber, 2002; Tapiero et al., 2004; Krinsky and Johnson, 2005; Rao and Rao, 2007).

Traditionally, the production of carotenoids in large scale has been developed, above all, from plant extracts or synthetic compounds (Maldonade et al., 2008). However, there is a growing demand for natural sources for the production of carotenoids that has driven efforts directed at reducing the costs involved in the production of pigments by means of fermentation processes in comparison to the techniques of extracting pigments from vegetable or synthetic sources (Dufossé, 2006; Kim et al., 2009; Ribeiro et al., 2012; Nguyen et al., 2012).

Sporobolomyces ruberrimus H110 is a red yeast that synthesizes β-carotene and, especially, the torularhodin, whereas both of them act as precursors to vitamin A (Fregova et al., 2004; Razavi et al., 2006). Since they are intracellular pigments, cell lysis is a major step toward the recovery and purification of these products. Cell lysis, or cell rupture and/or permeabilization, can be activated by mechanical or non-mechanical methods (physical, chemical and enzymatic) (Roh et al., 2008; Mayerhoff et al., 2008).

The mechanical methods are particularly efficient in the process of rupturing the cell's rigid wall present on red yeasts, which is typically required for the extraction of synthesized carotenoids by these microorganisms (Park et al., 2007; Ricci-Silva et al., 2000; Squina and Mercadante, 2003). On the other hand, wide varieties of chemical compounds have the ability of permeabilizing a microorganism's cell membrane, differing in the selectivity and efficiency for different species (Flores et al., 1994; Geciova et al., 2002).
The present study aimed to study different methods for cell lysis of the *Sporobolomyces ruberrimus* H110 yeast aiming at the recovery of intracellular pigments.

MATERIALS AND METHODS

Micro-organism

The *Sporobolomyces ruberrimus* H110 yeast, isolated at the 'Laboratoire des Réactions et Génie des Procédés' (LRGP), Nancy, France, was maintained in solid medium *Yeast Malt agar* (YM agar) at 4 °C.

Cultivation Medium

The YM agar cultivation medium was composed of: 10 g L^{-1} of glucose, 5 g L^{-1} of peptone, 3 g L^{-1} of yeast extract, 3 g L^{-1} of malt extract and distilled water to make 1 L, . Erlenmeyer flasks, containing 100 mL of cultivation medium were sterilized in autoclave at 121°C for 15 minutes. The pH was adjusted to 6 before sterilization and it was not controlled during the fermentation.

Fermentation

The *Erlenmeyer* flasks were inoculated with *Sporobolomyces ruberrimus* H110 and incubated at 27°C in shaker (Tecnal TE-421; Piracicaba, SP, Brazil) under an agitation of 150 rpm for 72 hours, reaching a cell concentration of approximately 6 g L^{-1}. The biomass was separated by centrifugation (Centribio® LDT80-2B centrifuge; São Paulo, SP, Brazil) at 5000 rpm for 10 minutes and recovered for the cell lysis experiments, and the supernatant was discarded.

Carotenoid extraction and analysis

Carotenois extraction

The biomass of *Sporobolomyces ruberrimus* H110 separated from the middle of fermentation was submitted to three methods of cell lysis for carotenoid extraction, being two mechanical methods (vortex and planetary mill) and a chemical method (dimethyl sulfoxide).
The extraction using vortex was performed in tube shaker (AP56 Phoenix; Araraquara, SP, Brazil). Added to test tubes were 500 mg of biomass of *Sporobolomyces ruberrimus* H110, 0.7 mL of petroleum ether and the lysis agent (irregular quartz stones - 6.3 mm; or glass beads - 2.3 mm) in quantities shown in the experimental design (Table 1). The test tubes were agitated in vortex for 10 minutes. After resting for 10 min, the mixture with the consistency of gel was diluted by the addition of 0.3 mL of acetone (Park et al., 2007), homogenizing the content in vortex. The analysis was performed after a new centrifugation process which was carried out in order to isolate the solvent solution and pigment.
For the extraction in planetary mill (Fritsch Pulverisette5 planetary mill; Idar-Oberstein -Germany), grinding jars or vases were used: accessories to the equipment employed in the present study that has volume of 250 mL and is composed of chromium steel (stainless). The lysis agents (irregular quartz stones - 6.3 mm or glass beads 2.3 mm) in the quantities determined by the experimental plan and the solvent for pigment extraction (4 mL of petroleum ether) were added to 500 mg of biomass of *Sporobolomyces ruberrimus* H110. The solution agitated to the speed which was also determined in the experimental plan, for 10 minutes. The analysis were performed after a new centrifugation process which was carried out in order to isolate the solvent solution and pigment In the chemical extraction with dimethyl sulfoxide (DMSO) glass test tubes were also employed, where the lysis agent, in this case DMSO in quantities defined by the experimental plan were added to 500 mg of biomass of *Sporobolomyces ruberrimus* H110. The solution was agitated in vortex, to speeds which were also determined in the experimental plan, for 10 minutes. After resting for 10 minutes, 1 mL of petroleum ether was added, once again using vortex agitation. After centrifuging the mixture, the solution was cloudy, which could compromise the subsequent analysis. For this reason, the content was washed with 1 mL of distilled water in order to allow the extraction of the soluble substances (Squina and Mercadante, 2003; Buzzini et al., 2005). After a new agitation process, the pigmented solution was isolated and then the tests were performed.
In all the methods studied, the extracted carotenoids were collected and temporarily stored until their analysis in test tubes wrapped with aluminum film in order to prevent the accelerated photo degradation of the pigment (Razavi et al., 2006; Buzzini et al., 2005; Moriel et al., 2007; Weber et al., 2007).

Experimental Design

An experimental factorial 2^2 design was performed with the application of 5 repetitions on the central point, later supplemented with 4 axial points to obtain a response surface subject to optimization. The independent factors analyzed were the concentration of the lysis agent (X_1) and the agitation speed (X_2) on the variable response (Y) of the total extracted carotenoids (Table 1).

Table 1. Levels of the independent factors and their coded values used in the studies on the extraction of pigments.

Variable[a]	Levels and range				
	-1.41	-1	0	+1	+1.41
(X_1) Concentration of the lysis agent (mg): glass beads and irregular quartz stones	71.6	90	135	180	198.5
(X_1) Concentration of the lysis agent (mL): DMSO	1.4	2	3.5	5	5.6
(X_2) Agitation speed (rpm) - vortex	430	800	1700	2600	2970
(X_2) Agitation speed (rpm) - "planetary mill"	60	100	200	300	340

[a]X_1: Lysis agent concentration and X_2 agitation.

The statistical analysis of the results was performed using the software 'Statistica®' version 7.0, by variance analyses (ANOVA), for 95% of the confidence interval.

Quantitative Analysis of the carotenoids extracted from yeast

The estimated quantification of the total extracted carotenoids, expressed in equivalents of torularhodin, was obtained from the maximum values of absorbance in a spectrophotometer (Shimadzu UV1601; Kyoto, Japan) at 480 nm and the specific extinction coefficient applied to the petroleum ether for the torularhodin of $\varepsilon^{1\%}_{1cm} = 2580$ [24]. The concentration of carotenoids was obtained by the equation 1 (Buzzini et al., 2007).

$$C_{carotenoides} = \frac{\lambda_{máx}.V.10^4}{\varepsilon^{1\%}_{1cm}.m} \quad \text{(Eq.1)}$$

Where λ_{max} is the maximum value of absorbance (nm), V is the volume of dilution (mL), $\varepsilon^{1\%}_{1cm}$ is the specific extinction coefficient for torularhodin; m is the mass of cells in the sample (g).

Cell rupture analysis

After the application of mechanical and chemical methods for the extraction of carotenoids, the *Sporobolomyces ruberrimus* H110 cells were placed on slides and viewed under an optical microscope (Olympus CX22; Hicksville, NY, USA). The morphological changes related to the cell lysis were documented on an Olympus BX41 (Hicksville, NY, USA) photomicroscope, composed by a Media Cybernetics camera, model Cool SNAP-Pro. The images were captured using the Pro-Plus 4.5 software (Media Cybernetics, Inc.; Rockville, MD, USA).

RESULTS AND DISCUSSION

Methods of mechanical and chemical extraction: vortex + lysis agent (glass beads, irregular quartz stones and DMSO)

The results (µg of carotenoids/g of cells) obtained from the central composite planning (2^2) demonstrated that the extraction method in which irregular stones were used presented lower values (0.33 µg of carotenoids g_{cells}^{-1}) to those obtained with the extractions using vortex and DMSO (1.31 µg of carotenoids g_{cells}^{-1}) or glass beads (1.07 µg of carotenoids g_{cells}^{-1}) (Table 2).

The degree of cell rupture in ball mills increases with the load of balls due to the increased ball-ball interaction (Geciova et al., 2002). The glass beads have virtually identical defined sizes (2.3 mm), formats (oval) and masses, whereas the irregular stones have varied sizes (6.3 mm), formats and masses. Thus, in many cases, few irregular stones were inserted (3 or 4 units) into the test tube, since the mass would be sufficient to achieve the lysis agent concentration as per the experimental plan. This fact influenced negatively the extraction of pigments due to the low stone-stone interaction. This is something that does not take place with the glass beads due to their dimensions and uniformity, since they admit a greater amount required of this lysis agent inside the test tube to achieve the values stipulated in the experimental plan when compared to the irregular stones.
In light of this analysis, the results obtained with the extraction using vortex + irregular stones were excluded from the statistical study. By contrast, the results obtained with the extraction using vortex + glass beads and vortex + DMSO showed a significant lack of adjustment (Tables 3 and 4).

Table 2. Results (µg of carotenoid g_{cells}^{-1}) obtained from the central composite planning (2^2) for mechanical and chemical extraction methods: vortex + lysis agent (glass beads or irregular quartz stones and DMSO).

			Glass beads	Irregular stones	DMSO
Experiment	**X_1**	**X_2**	**Y (µg carotenoids g_{cells}^{-1})**		
1	-1	-1	0.56	0.16	0.87
2	1	-1	0.66	0.28	1.24
3	-1	1	1.33	0.21	0.91
4	1	1	1.07	0.33	1.31
5	0	0	0.71	0.36	0.89
6	0	0	0.68	0.57	0.93
7	0	0	0.53	0.41	0.86
8	0	0	0.60	0.40	0.98
9	0	0	0.69	0.35	0.95

Experiments from 5 to 9 correspond to the central points.

Table 3. The regression coefficient values and its level of significance from the experimental data using vortex + glass beads for planning 2².

Regression coefficient	Value	p
β_0	0.758889	0.000001*
β_1	-0.040000	0.347929
β_2	0.295000	0.001433*
β_{12}	-0.090000	0.075130
Lack of adjustment	-	0.006487*

* ($p \geq 0.05$)

Table 4. The regression coefficient values and its level of significance from the experimental data using vortex + DMSO for planning 2².

Regression coefficient	Value	p
β_0	0.993333	0.000001*
β_1	0.192500	0.001274*
β_2	0.027500	0.312616
β_{12}	0.007500	0.768629
Lack of adjustment	-	0.007376*

* ($p \geq 0.05$)

Due to the lack of a significant adjustment reported (Tables 3 and 4), the factorial planning was complemented with 4 experiments at the axial points (–1.41 and 1.41) to create a central composite drawing that would generate an optimizing response surface, thus obtaining a new experimental matrix with 13 experiments (Table 5). The efficiency of chemical permeabilization in cells by means of solvents such as toluene, chloroform and acetone depends on its concentration in the solution (Geciova et al., 2002). The statistical analysis presented indicated that for the present study for extraction with DMSO (solvent) the concentration of the lysis agent is a statistically significant factor (Table 6). In regards to the extraction of carotenoids when glass beads are used, the speed is the one who carries a significant factor (Table 7).

This phenomenon takes place due to the fact that the extraction using DMSO does not require a high agitation speed, since the extraction does not depend on the friction of the solvent with the cells, but only in the contact of the solvent with the cell wall. As reported in a previous study in which the efficiency of the extraction process of carotenoids in *Rhodotorula graminis* is maximized when the cell solution with DMSO is incubated for 1 hour (Buzzini et al., 2005) and other studies that corroborate with the incubation of red yeast cells + DMSO for the extraction of carotenoids (Weber et al., 2007; Carnecká, 2009). The analysis of the regression coefficients of experiments (Tables 6 and 7) enabled the obtaining of the empirical models able to describe the effect of the independent variables, lysis agent concentration and agitation speed, in the variable response of the total number of extracted carotenoids. The empirical model is usually described by equations of second order: for the extraction with glass beads (Equation 2) and for extraction with DMSO (Equation 3).

$$Y = 0.65 + 0.16X_1^2 + 0.24X_2 + 0.1X_2^2 \quad \text{(Eq. 2)}$$

$$Y = 0.92 + 0.18X_1 + 0.18X_1^2 \quad \text{(Eq. 3)}$$

Using equations 2 and 3, it is possible to define the optimal combination of values X_1 and X_2 in order to obtain the maximization of the variable response (Y) (Table 8).
The achieved result corroborates with the data obtained in the literature. For a better extraction of intracellular byproducts, 10% of glass beads (mass/volume) (Carnecká, 2009) must be added and high loads in glass beads diminish the efficiency of the process, besides causing an increase in temperature and energy consumption, even if micro-organisms such as yeasts do not require high speeds for the cell disruption to be effective, since the size of these cells aid in increasing the contact of these with the glass beads (Villoutreix, 1960).

Table 5. Analytical responses from the central composite planning applied to the method of carotenoids extraction: vortex + lysis agent (glass beads and DMSO).

Variable	Experiments		Vortex	
			Glass beads	DMSO
	Lysis agent concentration	Agitation speed	Carotenoids ($\mu g/g_{cells}$)	
1	-1	-1	0.56	0.87
2	+1	-1	0.66	1.24
3	-1	+1	1.33	0.91
4	+1	+1	1.07	1.31
Ax1	-1.41	0	0.76	1.06
Ax2	+1.41	0	1.14	1.52
Ax3	0	-1.41	0.58	1.01
Ax4	0	+1.41	1.08	0.81
9	0	0	0.71	0.89
10	0	0	0.68	0.93
11	0	0	0.53	0.86
12	0	0	0.60	0.98
13	0	0	0.69	0.95

Table 6. Regression coefficient values of the central composite planning and its significance in the process of mechanical extraction method: vortex + glass beads.

Regression coefficient	Factor	p
B_0	0.64	0.000045*
B_1(L)	0.05	0.151948
B_{11}(Q)	0.16	0.005250*
B_2 (L)	0.24	0.000895*
B_{22}(Q)	0.10	0.026848*
β_{12}	0.09	0.075130
Lack of adjustment	-	0.072026

*(p ≥0.05)

Table 7. Regression coefficient values of the central composite planning and its significance in the process of mechanical extraction method: vortex + DMSO.

Regression coefficient	Factor	p
B_0	0.92	0.000002*
B_1(L)	0.18	0.000458*
B_{11}(Q)	0.18	0.000574*
B_2 (L)	-0.02	0.270294
B_{22}(Q)	-0.01	0.586115
β_{12}	0.007	0.768629
Lack of adjustment	-	0.145913

*(p ≥0.05)

Table 8. Decoded values of the optimum conditions for the extraction of carotenoids using the following extraction method: vortex + glass beads and vortex + DMSO with a concentration of 500 mg of cells and rupture time of 10 minutes.

Factor	Vortex + glass beads	Vortex + DMSO
Agitation speed	1230 rpm	Not significant
Lysis agent concentration	135 mg	2.7 mL

Method of mechanical extraction: planetary mill + lysis agent (glass beads and irregular quartz stones)
After the completion of the statistical study evaluating the extraction of carotenoids with different lysis agents in vortex, it was decided to apply the same experimental matrix using the planetary mill. For the physical capacity using this equipment, the higher speed of agitation that can be achieved is 340 rpm, but it presents a high degree of vibration which allows mitigating a loss of agitation speed and ensures a high interaction between the cell-lysis agent (Table 9).

Table 9. The values of carotenoid extraction using as extraction method: planetary mill + glass beads and planetary mill + irregular quartz stones with a cell concentration of 500 mg and rupture time of 10 minutes.

Lysis agent	Lysis agent concentration	Carotenoid concentration
Glass beads	135 mg	1.74 µg/g cell
Irregular quartz stones	135 mg	1.57 µg/g cell

Unlike the limitation related to the charge of lysis agents found in the method that employs test tubes in vortex, in the use of the planetary mill equipment, similar satisfactory extraction results will be achieved, both with the use of glass beads as with the use of irregular stones. It is likely that these results are justified by the proportional increase in the total volume of processed samples planetary mill on the grounds of greater volumetric capacity of the milling containers (250 mL) when compared with the test tubes (8 mL) employed in the extraction method with vortex.
The use of the planetary mill equipment for the extraction of intracellular pigments has also shown promising results, as it presents several advantages when compared to other extraction methods. One of them is the possibility of using the milling containers with an inert atmosphere, thus preventing pigment degradation due to the action of the atmospheric air (if compared to extraction with glass beads + vortex), and the non-usage of solvents, which are generally toxic (when using DMSO). Another advantage is the possibility of regulating the milling power for cell rupture, thus avoiding material heating, and ensuring a high efficiency of cell rupture and minimizing pigment degradation.
The kinetic energy that is achieved in the planetary mill is much larger than the one reached on a vortex, where there is almost no energy transfer to the spheres. In the planetary mill, a high energy milling process takes place, where the centripetal force produced by the rotation of the container on its own axis and by the rotation of the support disk acts on the contents of the container, causing the substance in its interior to be crushed by the balls. In the vortex, when the agitation speed is increased above a critical level, the action of the centripetal force tends to stick on the walls, forming the so-called 'rolling paths' where there is no resulting ball impact, which impairs the milling (Villoutreix, 1960).
Considering that the use of the planetary mill with four milling containers, the use of the planetary mill can be considered as economically advantageous in face of the use of a tube shaker in *Sporobolomyces ruberrimus* H110 cell lysis under the studied conditions.
Positive results were found in relation to the use of this type of equipment for cell lysis, which produced yields close to 100% for microalgae cells tested under any studied conditions (diameter and material of the lysis agents). Two 4-minute cycles at 400 rpm were applied to the samples (10 g of cells in 30 mL of ethanol) with a resting interval of 1 minute. According to the comparative assessment, such extraction method presents cell lysis rates, and positive technical reproducibility and operation costs (Carnecká, 2009).

Cell rupture/permeabilization

As observed in Figure 1 (a), the original format, before any yeast treatment, is oval. After the mechanical treatment (Figure 1 (b)) the cells were ruptured or fragmented and, at the analyzed sample, it was not possible to observe any intact cells. The presence cell 'debris' in Figure 1 (b) points to the fact that total cell laceration is more often when mechanical extraction methods are applied, mainly when glass beads are used, possible due to their dimension (2.3 mm) (Mayerhoff et al., 2008; Geciova et al., 2002).

The use of a chemical agent to the recover the pigments, despite not having achieved total carotenoid extraction, DMSO resulted in pigment extraction. Observing Figure 1 (c), it is believed that there was only cell permeabilization, since the cell was apparently remained intact after cell treatment. This result corroborates with those obtained in the literature, where treatment with DMSO does not result in the rupture of treated cells (Gu et al., 2008; Khodaiyan et al., 2008).

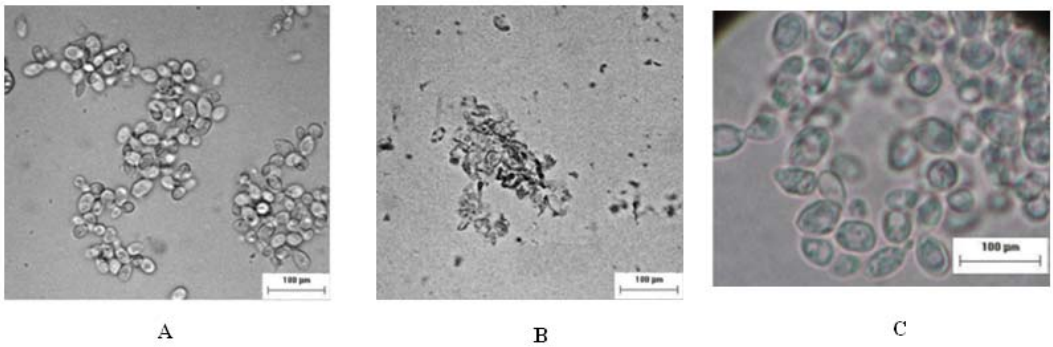

Figure 1- Aspect of *Sporobolomyces ruberrimus* cell structure (A) before extraction; (B) after extraction treatment of the carotenoids by mechanical methods using glass beads and (C) after chemical treatment with DMSO.

CONCLUSION

We conclude that for the evaluated standards and methods, the method that was the most promising was the mechanical one with the use of the planetary mill equipment. As for cell lysis, the direct microscopic analysis and the quantification of the intact cells indicated that the studied mechanical methods for cell lysis proved to be more efficient in the sense of cell laceration and that carotenoid cell extraction by applying the chemical method with DMSO only takes place, possibly, by cell permeabilization.

REFERENCES

Buzzini P., Innocenti M., Turchetti B., Libkind D., Broock M.V. and Mulinacci N. (2007). Carotenoid profiles of yeasts belonging to the genera *Rhodotorula*, *Rhodosporidium*, *Sporobolomyces* and *Sporidiobolus*. Microbiology: Vol.53, 1024-1031

Buzzini P., Martini A., Gaetani M., Turchetti B., Pagnoni U.M. and Davioli P. (2005). Optimization of carotenoid production by *Rhodotorulagraminis* DBVPG 7021 as a function of trace element concentration by means of response surface analysis. Enzyme Microbial Technology: Vol.36, 687-692

Čarnecká M. (2009). Molecular study of intracellular changes as response of microorganisms to environment. Ph.D. Thesis. Brno University of Technology, Brno, Czech Republic.

Davoli P. and Weber R.W.S. (2002). Carotenoid pigments from the red mirror yeast, *Sporobolomycesroseus*. Mycologist: Vol.16, 102-108

Dufossé L. (2006) Microbial production of food grade pigment. Food Technology Biotechnology: Vol.44, 313-321

Flores M.V., Voget C.E. and Ertola R.J.J. (1994). Permeabilization of yeasts cells (*Kluyveromyceslactis*) with organic solvents. Enzyme and Microbial Technology: Vol.16, 340-346

Fregova G.I., Simova E.D. and Beshkova D.M. (2004). Improvement of carotenoid-synthesizing yeast *Rhodotorularubra* by chemical mutagenesis. Verlag der Zeitschrift für Naturforschung: Vol.59c, 99-103

Geciova J., Bury D. and Jelen P. (2002). Methods for disruption of microbials cells for potencial use in the dairy industry – a review. International Dairy Journal: Vol.12, 541-553

Gu Z., Chen D., Han Y., Chen Z. and Gu F. (2008). Optimization of carotenoids extraction from *Rhodobacter sphaeroides*. Food Science and Technology International: Vol.41, 1082-1088

Iturriaga E.A., Velayos A. and Eslava A.P. (2000). Structure and Function of the Genes Involved in the Biosynthesis of Carotenoids in the Mucorales. Biotechnology and Bioprocess Engineering Journal: Vol.5, 263-274

Khodaiyan F., Razavi S. H. and Mousavi S. M. (2008). Optimization of canthaxanthin production by *Dietzianatronolimnaea* HS-1 from cheese whey using statistical experimental methods. Biochemical Engineering Journal: Vol.40, 415–422.

Kim J.H., Kim S.W., Nguyen Q.A., Li H., Kim S.B., Seo Y.G., Yang J.K., Chung I.Y., Kim D.H. and Kim C.J. (2009). Production of β-carotene by Recombinant *Escherichia coli* with Engineered Whole Mevalonate Pathway in Batch and Fed-batch Cultures. Biotechnology Bioprocess Engineering: Vol.14, 559-564

Krinsky N.I. and Johnson E.J. (2005). Carotenoid actions and their relation to health and disease. Molecular Aspects of Medicine: Vol.26, 459-516

Maldonade I.R., Rodriguez-Amaya D.B. and Scamparini A.R.P. (2008). Carotenoids of yeasts isolated from the Brazilian ecosystem. Food Chemistry: Vol.107, 145-150

Mayerhoff Z.D.V.L., Franco T.T. and Roberto I.C. (2008). A study of cell disruption of *Candida mogii* by glass bead mill for the recovery of xylose reductase. Separation and Purification Technology: Vol.63, 706–709

Moriel D.G., Chociai M.B., Machado I.M.P., Fontana J.D. and Bonfim T.M.B. (2005). Effect of feeding methods on the astaxanthin production by *Phaffiarhodozyma* in fed-batch process. Brazilian Archives of Biology and Technology: Vol.48, 397-401

Nguyen A.Q., Kim S.W., Kim S.B., Seo Y.G., Chung I.Y., Kim D.H. and Kim G.J. (2012). Production of β-carotene and Acetate in Recombinant *Escherichia coli* with or without Mevalonate Pathway at Different Culture Temperature or pH. Biotechnology Bioprocess Engineering: Vol.17, 1196-1204

Park P.K., Kim E.Y. and Chu K.H. (2007). Chemical disruption of yeast cells for isolation of carotenoids pigments. Separation and Purification Technology: Vol.53, 148-152

Rao A.V. and Rao L.G. (2007). Carotenoids and human health. Pharmacological Research: Vol.55, 207-216

Razavi S.H., Blanchard F. and Ivan M. (2006). UV-HPLC / APCI-MS Method for separation and identification of the carotenoids produced by *Sporobolomycesruberrimus* H110. Iranian Journal of Chemistry and Chemical Engineering: Vol.25, 1- 10

Reyes L.H., Gomez J.M. and Kao K.C. (2014). Improving carotenoids production in yeast via adaptive laboratory evolution. Metabolic Engineering: Vol.21, 26–33

Ribeiro B.D., Coelho M.A.Z. and Barreto D.W. (2012). Production of concentrated natural beta-carotene from buriti (*Mauritia vinifera*) oil by enzymatic hydrolysis. Food and Bioproducts Processing: Vol.90, 141–147

Ricci-Silva M.E., Vitolo M. and Abrahão-Neto J. (2000). Protein and glucose 6-phospate dehydrogenase releasing from baker's yeast cells disrupted by a vertical bead mil. Process Biochemistry: Vol.35, 831-835

Roh M.K., Uddin M.S. and Chun B.S. (2008). Extraction of Fucoxanthin and Polyphenol from *Undaria pinnatifida* Using Supercritical Carbon Dioxide with Co-solvent. Biotechnology Bioprocess Engineering: Vol.13, 724-729

Squina F.M. and Mercadante A.Z. (2003). Análise, por CLAE, de carotenoides de cinco linhagens de *Rodotorula*. Revista Brasileira Ciência Farmacológica: Vol.39, 309- 318

Tapiero H., Townsend D.M. and Tew K.D. (2004). The role of carotenoids in the prevention of human pathologies. Biomedicine & Pharmacotherapy: Vol.58, 100-110

Villoutreix J. (1960). Les carotenoides de *Rhodotorula mucilaginosa*étude de leurbiosynthèse à l´aide de l´analyse de mutantset de l´emploi d´uninhibiteur de lacaroténogénèse. Biochimica et Biophysica Acta: Vol.40, 442-457

Weber R.W.S., Anke H. and Davioli P. (2007). Simple method for the extraction and reversed-phase high-performance liquid chromatographic analysis of carotenoid pigments from red yeasts (Basidiomycota, Fungi). Journal of Chromatography A: Vol.1145, 118-122

ISSN : 0976-4550

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Email : ijabpt@gmail.com

Website: www.ijabpt.com