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Original article

Optimisation of *Spirulina platensis* convective drying: evaluation of phycocyanin loss and lipid oxidation

Elizangela G. Oliveira, Jessica H. Duarte, Kelly Moraes, Valeria T. Crexi & Luiz A. A. Pinto*

Unit Operations Laboratory, School of Chemistry and Food, Federal University of Rio Grande (FURG), P.O. Box 474, Zip 96201-900, Rio Grande, RS, Brazil

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Summary The aim of the study was the optimisation of *Spirulina platensis* drying on convective hot air through the response surface methodology. The responses were thiobarbituric acid (TBA) and phycocyanin loss percentage values in final product. Experiments were carried out in perforated tray drier with parallel air flow, and the wet samples thickness and drying air temperatures were in range of 3–7 mm and 50–70 °C, respectively. The statistical analysis showed significant effect ($P < 0.05$) for air temperature and samples thickness. In the best drying condition, 55 °C and 3.7 mm, presented the phycocyanin loss percentage and the TBA values of approximately 37% and 1.5 mg_{MDA} kg^{-1}, respectively. In this drying condition, the fatty acids composition of the microalgae *Spirulina* did not show significance difference ($P > 0.05$) in relation to fresh biomass. The lipid profile of dried product presented high percentage of polyunsaturated fatty acids (34.4%), especially the gamma-linolenic acid (20.6%).

Keywords Drying, fatty acids, microalgae, phycocyanin, spirulina platensis, thiobarbituric acid.

Introduction

The microalgae *Spirulina platensis* is produced commercially all over the world, and the dried product is valuable food supplement. It is rich in proteins (60–70% by dry weight), vitamins (especially B_{12} and β-carotene) and minerals. It contains many essential amino acids and fatty acids (Jiménez *et al*., 2003); also it is an inexpensive source of pigment (Richmond, 1988). The *Spirulina* components, with antioxidants properties, are the polyunsaturated fatty acids and pigments (Estrada *et al*., 2001). Phycocyanin and gamma-linolenic acid (C18:3, ω6, GLA) are the components of the *Spirulina*, which has been receiving more attention from researchers.

Phycocyanin is the main pigment produced by the microalgae *Spirulina platensis* and reaches 20% in dry weight of the cell protein (Vonshak, 1997). Phycocyanin has a significant antioxidant, anti-inflammatory, hepatoprotective and free radical properties; it is also used in food colouring and in cosmetics as they are nontoxic and noncarcinogenic (Henrikson, 1994; Morist *et al*., 2001; Minkova *et al*., 2003;). Phycocyanin is used in chewing gums, dairy products, ice creams, jellies (Cohen, 1986; Yoshida *et al*., 1996) and biomedical research (Glazer, 1994). It is used as potential therapeutic agent reducing the oxidative stress disease (Romay *et al*., 1998; Bhat & Madyastha, 2001). The extraction method of phycocyanin from *spirulina* biomass is suggested by several methods such as spray drying and oven dried which result in approximately 50% loss of phycocyanin (Sarada *et al*., 1999). The improved drying method is important to store maximum amount of phycocyanin in the biomass (Doke, 2005).

The microalgae *Spirulina platensis* is a potential source of gamma-linolenic acid (GLA), an essential polyunsaturated fatty acid with economic interest. GLA is metabolite of linolenic acid (LA) and the first intermediate in the conversion of LA to arachidonic acid (AA) (Gustone, 1992). The microbial production for extraction of polyunsaturated fatty acid (PUFA) is considered an economical alternative to produce large quantities of fatty acids (Kennedy *et al*., 1993).

Dehydration operation is an important step in the chemical and food processing industries. The basic objective in foodstuffs drying is the removal of water in the solids for to minimise the microbial growth and deterioration by chemical reactions (Krokida *et al*., 2003). Also, it leads the reduction in weight and volume of material, storage and transportation costs (Okos *et al*., 1992). Convective hot air drying is one of the most

*Correspondent: Fax: +55 53 3233 8745;
e-mail: dqmpinto@furg.br; luiz.pinto@pq.cnpq.br

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common industrial methods used for drying organic material and is a simultaneous process that involves heat and mass transfer followed by a phase or state change (Babalis & Belessiotis, 2004).

The drying of *Spirulina platensis* constitutes approximately 30% of the total production cost, and the traditional methods used to process fresh biomass into dry *Spirulina* are spray drying, freeze drying, solar drying, convective hot air drying and spouted bed (Morist *et al*., 2001; Jiménez *et al*., 2003; Desmorieux & Decaen, 2006; Oliveira *et al*., 2009). Sarada *et al*. (1999) found a loss of approximately 50% of the phycocyanin presented in the biomass when different techniques have been used to dry microalgae *Spirulina*, including the spray and convective dryers. Oliveira *et al*. (2008) reported that high drying temperature (> 60 °C) in two different dryers (spouted bed and convective air dryers) decreased the amount of phycocyanin extractable from *Spirulina platensis*. When low-cost drying methods are optimised, e.g. convective dryer, have the potential to approach the advantages derived from spray drying in terms of quality and bioavailability at lower cost.

Several factors can influence hot air drying of foods, for example: velocity and temperature of air, water diffusion through material, load density, thickness and shape of the product. The factors temperature and thickness are more important in the microalgae drying operation according to literature (Desmorieux & Decaen, 2006; Oliveira *et al*., 2009). However, the water removal decreases the nutritional and sensorial values of food and leads to phenomena such as hardening and shrinkage (Vega *et al*., 2007). The microalgae *Spirulina platensis* is a high source of biocompounds, as phycobiliproteins (phycocyanin) and essential fatty acids; it is important reason to optimise the drying conditions.

The aim of this study was the optimisation of the of microalgae *Spirulina platensis* drying through the response surface methodology (RSM), considering as independent variables the drying air temperature and sample thickness. The responses were phycocyanin loss percentage and thiobarbituric acid (TBA) values. The fatty acids profile was determined in the best drying condition and compared with the fresh biomass.

Materials and methods

Raw material

Spirulina strain LEB-18 (Costa *et al*., 2004) was cultivated in a 450 L open outdoor photo-bioreactors, under uncontrolled conditions, in the south of Brazil. During these cultivations, water was supplemented with 20% Zarrouk synthetic medium (Zarrouk, 1966), containing (g L^{-1}): $NaHCO_3$, 16.8; $NaNO_3$, 2.5; K_2HPO4, 0.5; K_2SO_4, 1.0; NaCl, 1.0; $MgSO_4.7H_2O$, 0.2; $CaCl_2$, 0.04; $FeSO_4.7H_2O$, 0.01; EDTA, 0.08 and micronutrients. The initial biomass concentration was 0.15 g L^{-1}. Samples were taken every 24 h to determine the biomass concentration by optical density measurements at 670 nm using a spectrophotometer (Quimis model Q108-DRM, São Paulo, Brazil). In the end of cultivation, the biomass was recovered by filtration and pressing.

Biomass drying

Drying experiments were carried out at 50, 60 and 70 °C in the discontinuous tray dryer. The samples thicknesses were 3, 5 and 7 mm with load density of 4 kg m^{-2}, and the hot air velocity was 2.5 m s^{-1}. The drying procedure was continued till the moisture content of the sample was about 0.10 kg kg^{-1} (wet basis). After drying experiments, all products were ground in a knife mill (Willey model, Philadelphia, USA) and sieved (150 mesh), packed in plastics bags and stored at ambient temperature. Each drying experiment was carried out in duplicate.

Chemical analyses

The following parameters were determined in duplicate for the *Spirulina platensis* fresh: moisture (method 925.10), protein (method 960.52) and ash (method 923.03) contents. Determinations were carried out according to Association of Official Analytical Chemists, AOAC, (1995). Lipids content was through the methodology proposed by Folch & Lees (1957), and carbohydrate content was determined by difference.

Phycocyanin content

Quantitative analysis of phycocyanin was carried out by Spectrometric method, according to Boussiba & Richmond (1979), on fresh and dried biomass. Initially, it was added 2 g of the wet sample in the pan, and after, it was dried in the oven for 6 h. To determine the percentage of phycocyanin, 40 mg of dried *Spirulina* was weighed, mixed in 10 mL of phosphate buffer 0.1 M (pH = 7) and stirred until complete dissolution. The samples were stored in refrigerator at 4 °C overnight. The samples were subsequently mixed and centrifuged (Fanem model Baby I 206BL, São Paulo, Brazil) at 10 °C, 4000 ***g*** for 5 min. The absorbance was read in spectrophotometer (Quimis model Q-108DRM, São Paulo, Brazil) at 620 nm using phosphate buffer as blank. Phycocyanin content was calculated according to Eqn 1:

$$\%\ \text{Phyco} = \frac{A_{620}.\text{nd}}{3.39.(m_{\text{sample}}).(X_{\text{dry matter}})}.100 \qquad (1)$$

where % Phyco is the phycocyanin percentage in sample, A_{620} is the absorbency in a wave length of

620 nm, nd is the dilution number (mL), 3.39 is the coefficient of extinction for phycocyanin at 620 nm, m_{sample} is the wet mass of *Spirulina* (g), $X_{dry\ matter}$ is the *Spirulina* dry composition (dimensionless) and 100 is the representative of 100%. Each analysis was performed in duplicate.

TBA value

Dried samples were carried out to the TBA determination of lipid oxidation. The TBA value was done according to Tiburcio *et al.* (2007) with some modifications: *Spirulina* powder (10 g) was mixed with 40 mL of chloroform and filtrated. The filtrate (10 mL) was placed in tubes of centrifuge (Fanem model Baby I 206BL, São Paulo, Brazil) with 10 mL of trichloroacetic acid (TCA) 10% w/v and centrifuged at 2000 ***g*** for 15 min. The supernatant (4 mL) and 1 mL of thiobarbituric acid (TBA) 0.02 M were stirred for 5 min and then incubated in boiling water bath for 40 min to develop the colour. Absorbance of the resulting supernatant was determined at 530 nm by a spectrophotometer (Quimis model Q-108DRM, São Paulo, Brazil). The reagents used were of analytical grade. The TBA value was calculated through standard curve obtained by reacting of tetramethoxypropane 0.01 M with TBA, the value was expressed as milligrams of malonyldialdehyde (MDA) per kg of sample in dry basis.

Fatty acids profiles

To evaluate the fatty acids profiles of the microalga Spirulina platensis, lipids extraction was carried out by a methodology proposed by Folch & Lees (1957). The extraction was carried out in the fresh and dehydrated biomass. Fatty acid identification and quantification were carried out by chromatographic analysis for *Spirulina* oil. Fatty acids profiles were determined by preparation of methyl esters as described by Metcalfe & Schimitz (1966).

Fatty acid methyl esters (FAME) were identified by gas chromatography (chromatographer model Varian-3400 CX, Palo Alto, USA) equipped with a DB-17 J&W Scientific (50% phenyl methylpolysiloxane) capillary column. Fatty acid esters analyses were carried out in duplicate by injecting 1.0 μL, SPLIT ratio 1:50, into the capillary column (30 × 0.25 mm, 0.25 m film in thickness). GC setting conditions were as follows: injection temperature 250 °C and flame ionisation detector temperature 300 °C, helium gas carrier flow rate 1.0 mL min^{-1}, linear speed 24 cm s^{-1} and oven temperature held at 100 °C for 1 min, then increased to 160 °C at 6 °C min^{-1} and held at 230 °C at 6 °C min^{-1}. FAME were identified by direct comparison of the retention times with standards (SUPELCO™ 37, Bellefonte, PA, USA) and were quantified as the percentage area of each FAME mixture.

Statistical analysis

In the drying experiments, the effects of the drying air temperature (X_1) and the samples thickness (X_2) were studied through a full factorial design (3^2) on the responses for phycocyanin loss percentage and TBA values. The optimisation procedure was by the response surface methodology (Myer, 1976).

The independent variables (factors) investigated in this study were drying air temperature and wet samples thickness. The values of these factors were 50, 60 and 70 °C for air temperature; 3, 5 and 7 mm for samples thickness. The coded levels of these values, for both factors, were represented by (−1), (0) and (+1), respectively.

For the regression analysis of the experimental data, the software Statistica 6.0 for Windows (StatSoft Inc., USA) was used. The statistical significance of the second-order statistical model (Eqn 2) and the variance explained by this model were determined by Fisher's test and coefficient of determination (R^2), respectively. The response surfaces were found to define the optimal drying conditions for phycocyanin loss percentage and TBA values.

The second-order statistical model is presented in Eqn 2:

$$Y_n = b_0 + b_1X_1 + b_{11}X_1^2 + b_2X_2 + b_{22}X_2^2 + b_{12}X_1X_2 \quad (2)$$

where Y_n is the predicted response (in real value), X_1 is the codified temperature, X_2 is the codified thickness and b_0, b_1, b_{11}, b_2, b_{22} and b_{12} are the regression coefficients.

The fatty acids profile was found in the best drying condition, and the results were compared using the Tukey test at the significance level of 95% ($P < 5\%$), with the aid of the software Statistica 6.0 for Windows (Statsoft Inc., USA).

Results and discussion

Characterisation of the *Spirulina platensis* fresh was (in wet basis): moisture content 75.7% ± 0.2; ash content 1.7% ± 0.2; protein content 11.9% ± 0.3; lipids content 3.4% ± 0.5 and carbohydrate content 7.3% ± 0.1 (by difference). The phycocyanin content value of the fresh biomass was 0.11 kg kg^{-1}. The phycocyanin loss percentage values on dried products were calculated in relation to the fresh biomass. Table 1 shows the total drying time, phycocyanin loss percentage and TBA values of the factorial design matrix.

The TBA value is extensively used like an indicator to the lipid oxidation degree, quantified the secondary products by oxidation. According to Boran *et al.* (2006), the TBA value required for acceptability of oils for

Table 1 Results of the 3^2 factorial design matrix for drying experiments

Experiment (*n*°)	X_1 (Coded temperature)	X_2 (Coded thickness)	Total drying time* (min)	TBA value* (mg_{MDA} kg^{-1})	Phycocyanin loss percentage* (%)
1	−1	−1	192 ± 9	1.43 ± 0.10	43.76 ± 0.11
2	0	−1	150 ± 7	2.12 ± 0.03	43.84 ± 0.17
3	1	−1	133 ± 12	2.27 ± 0.15	75.30 ± 0.12
4	−1	0	369 ± 5	1.20 ± 0.01	56.24 ± 0.20
5	0	0	210 ± 5	0.90 ± 0.02	40.17 ± 0.15
6	1	0	145 ± 15	0.96 ± 0.05	76.15 ± 0.21
7	−1	1	608 ± 10	0.88 ± 0.04	82.22 ± 0.13
8	0	1	300 ± 6	0.76 ± 0.01	85.13 ± 0.14
9	1	1	214 ± 5	0.65 ± 0.05	93.07 ± 0.12

MDA, malonyldialdehyde; TBA, thiobarbituric acid.
*Mean value ± standard error (in replicate).

human consumption is in the range 7–8 mg_{MDA} kg^{-1}. Therefore, the oils obtained by dried *Spirulina* in all drying conditions (Table 1) presented oxidative quality. Drying process is a very important step to store maximum phycocyanin content in biomass and is an efficient extraction method for maximum recovery with relatively high purity ratio (Doke, 2005). According to Sarada *et al*. (1999), considerable loss of phycocyanin concentration was observed when wet biomass was dried at elevated temperature. The same occurred with the biomass dried at 70 °C and in the thickness of 7 mm (Table 1).

Statistical analysis of the results in Table 2 showed that in the drying of microalgae *Spirulina platensis* in convective air drying, both the study factors (air temperature and sample thickness) have a significance effect at level of 95% ($P < 0.05$) on the loss of phycocyanin and TBA values. The results in Table 2 show that the temperature has an important role on phycocyanin content during the drying process of *Spirulina* biomass. Also, it can be observed that the thickness presented significance in the loss of pigment, it can be explained due the higher thickness material had taken a larger time to dry (Table 1) until the commercial moisture content.

In Table 2, the results of TBA values show that the effects of the thickness were higher than temperature one in this response. The lipid oxidations do not occur in saturated fatty acids, only in drastic conditions of temperature. However, the TBA value in the higher temperatures and thickness showed a reduction, it can be explained due the drying experiments occurred faster in highest temperature, and also in the higher thickness, the oil was more protected of the action of the temperature. The results of the second-order statistical model of analysis of variance are shown in Table 3.

In Table 3, the F-test leads to that the model was adequate because the calculated (F_c) values were 3.48 and 3.25 times higher than the table (F_t) values for phycocyanin loss percentage and TBA values, respectively, at 95% of confidence. As a practical rule, a model has statistical significance when the calculated F value is at least 3–5 times higher than the table value (Khury & Cornell, 1996). These values guarantee a satisfactory fit of the quadratic models to the experimental data, Eqns 3 and 4, and indicated that 95% of the variability in the responses could be explained by the models. The coefficients of correlation (R^2) for phycocyanin loss percentage and TBA responses were 0.95 and 0.94, respectively.

Table 2 Effects and significance estimated by nonlinear regression

	Phycocyanin loss (%)				TBA value (mg_{MDA} $kg^{-1}_{dry\ sample}$)			
	Effect	Pure Error	t(2)	*P*	Effect	Pure Error	t(2)	*P*
Average	43.85	0.085	514.25	<0.05	0.96	0.006	163.41	< 0.05
X_1	20.76	0.136	153.00	<0.05	0.12	0.009	12.50	< 0.05
X_{11}	34.09	0.208	163.21	<0.05	0.03	0.015	2.30	0.148
X_2	32.50	0.136	239.49	<0.05	−1.18	0.009	−125.38	< 0.05
X_{22}	30.67	0.208	146.83	<0.05	0.75	0.015	51.50	< 0.05
X_{12}	−10.34	0.166	−62.23	<0.05	−0.53	0.011	−46.08	< 0.05

MDA, malonyldialdehyde; TBA, thiobarbituric acid; X_1, temperature linear effect; X_{11}, temperature quadratic effect; X_2, thickness linear effect; X_{22}, thickness quadratic effect; X_{12}, interaction effect.

Table 3 Analysis of variance (ANOVA) for responses of the statistical model

	SS	df	MS	F_c	F_t*	F_c/F_t
	Phycocyanin loss percentage (%)					
Regression	4153.01	5	830.60	17.59	5.05	3.48
Residual	236.07	5	47.21	–	–	
Total	4389.08	10	–	–		
	TBA value ($mg_{MDA}\ kg^{-1}{}_{drysample}$)					
Regression	2.78	5	0.55	14.75	4.53	3.25
Residual	0.18	5	0.04	–	–	
Total	2.97	10	–	–		

SS, Sum square; *df*, degree free; MS, mean square; MDA, malonyldialdehyde; TBA, thiobarbituric acid; F_c, calculated value; F_t, table value.
*The Fisher *F* distribution at 5% probability ($P < 0.05$).

$$Y_1 = 43.85 + 10.38X_1 + 17.04X_1^2 + 16.25X_2 + 15.33X_2^2 - 5.17X_1X_2 \quad (3)$$

$$Y_2 = 0.96 + 0.06X_1 - 0.59X_2 + 0.37X_2^2 - 0.26X_1X_2 \quad (4)$$

where Y_1 is phycocyanin loss percentage, in %; Y_2 is the TBA value, in $mg_{MDA}\ kg^{-1}{}_{sample}$; X_1 is the codified temperature; X_2 is the codified thickness.

The response surface curves and the contour plot for drying of microalgae *Spirulina platensis* were obtained by statistical model (Eqns 3 and 4) and are show in Figs 1 and 2. The drying condition with the lowest phycocyanin loss percentage value was considered the best condition. Figures 1 and 2 show that the lower phycocyanin loss percentage and TBA values were obtained in air temperature 55 °C and sample thickness of 3.7 mm. In the best drying condition, the responses for the phycocyanin loss percentage and TBA values were 37.4 ± 0.3% and 1.5 ± 0.1 $mg_{MDA}\ kg^{-1}$sample, respectively.

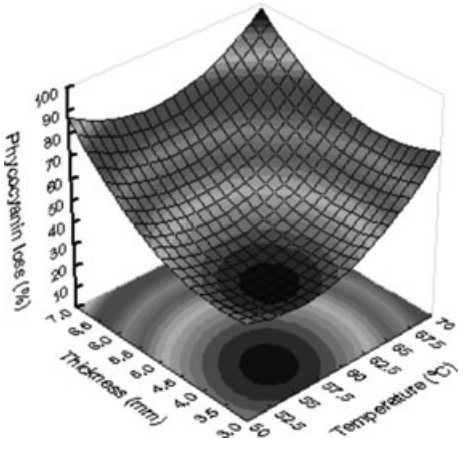

Figure 1 Response surface curve for loss of phycocyanin.

Tiburcio *et al*. (2007) studied the lipid oxidation of *Spirulina platensis* in three drying techniques, sun, solar and draft oven and compared with control sample dried in spray dryer. The lipid oxidation was evaluated through of TBA value. The authors found that the combined effect of tertiary-butyl hydroquinone (TBHQ) and microwave blanching was the most effective pre-dehydration treatment for minimising lipid peroxidation in drying *Spirulina*, and the lowest TBA value in product dried in sun-drying (0.47 $mg_{MDA}\ kg^{-1}$) also was closest to the spray-dried sample (control), 0.43 $mg_{MDA}\ kg^{-1}$. They suggest that sun-drying when optimised produced a dried product more stable than spray-dried. As the pre-dehydration treatment was not used in this study, the higher TBA values were observed (Table 1).

The drying method used in this study was considered satisfactory by statistical analysis in the drying of microalgae *Spirulina*, and in the best drying condition, the loss of phycocyanin was about 40%. This value was lower than those reported by the literature using methods more expensive and traditional of drying of microalgae (Sarada *et al*., 1999).

Table 4 shows the composition of main fatty acids present in microalgae *Spirulina platensis* fresh and after the drying process in the best condition. There were not significance differences ($P < 0.05$), through the Tukey test, between the oils obtained by *Spirulina* fresh and by dried product in the best drying condition. The oil obtained from the *Spirulina* is an important source of monosaturated and polyunsaturated fatty acids (MUFA and PUFA), about 51% of total fatty acids. In both, oils was found linoleic acid and linolenic acid about 13.9% and 20.6%, respectively. Thermal processes, as well as light exposure, may affect MUFA and PUFA contents because of the oxidation process (Morist *et al*., 2001). In Table 4, the drying process does not change the chemical structure of linolenic acid and also did not occur unfavourable transformations to the nutrients of this microalgae.

The fatty acids are susceptible molecules to treatments that use high temperature, leading to chemical

Table 4 Fatty acids compositions of microalgae *Spirulina* fresh and dried product

Fatty Acid (%)	*Spirulina* fresh*†	*Spirulina* dried*†
C11:0	3.50 ± 0.01[a]	3.48 ± 0.02[a]
C16:0	32.50 ± 0.02[a]	32.53 ± 0.01[a]
C16:1ω7	13.09 ± 0.02[a]	13.10 ± 0.02[a]
C18:0	2.89 ± 0.01[a]	2.90 ± 0.01[a]
C18:1ω9	3.10 ± 0,02[a]	3.20 ± 0.02[a]
C18:2 ω6 trans	13.88 ± 0.01[a]	13.86 ± 0.02[a]
C18:3ω6 cis	20.60 ± 0.02[a]	20.56 ± 0.01[a]
Saturated	38.89 ± 0.01[a]	38.91 ± 0.01[a]
Monounsaturated	16.19 ± 0.02[a]	16.30 ± 0.02[a]
Polyunsaturated	34.48 ± 0.01[a]	34.42 ± 0.01[a]
PUFA/SFA	0.88 ± 0.02[a]	0.88 ± 0.02[a]

*Tukey test: same letters indicate no significance differences ($P > 0.05$).
†Values means ± standard deviation (in replicate).

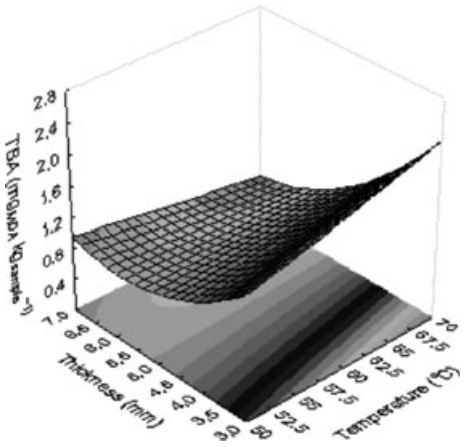

Figure 2 Response surface curve for TBA values.

transformations, polymerisation, geometrical isomerisation and intramolecular cyclisation (Berdeaux *et al*., 2007; Ceriane & Meirelless, 2007); however, in this best condition, the trans-linolenic fat acid was not changed after the drying process. A similar result was reported by Zepka *et al*. (2007), on drying the microalgae *Aphanothece microscopica Nägeli*, with different conditions in convective air dryer.

A higher content of PUFA increases the nutritional value of foods. The recommendation for the human diet is a polyunsaturated/saturated (PUFA/SFA) ratio higher than 0.45 (Cuthbertson, 1989). An analysis of Table 4 shows that the lipids profile of the microalgae *Spirulina* presents the (PUFA/SFA) ratio of 0.88. This result agrees with other authors (Campanella *et al*., 1999; Tokusoglu & Ünal, 2003) that considered the microalgae *Spirulina* as potential sources of the essential fatty acid gamma-linolenic acid.

Conclusion

The *Spirulina platensis* convective hot air drying, in thin layer with parallel air flow, showed a significant effect of air temperature and samples thickness ($P < 0.05$) on phycocyanin loss percentage and TBA values. Drying optimisation through the response surface methodology presented the best condition at air temperature of 55 °C and sample thickness 3.7 mm. In this condition, the responses values were of 37% for phycocyanin and 1.5 $mg_{MDA}\ kg^{-1}{}_{dry\ sample}$ for TBA value, respectively.

The best drying condition did not influence the fatty acids composition of final product in relation to fresh biomass, and it was verified by Tukey test at 95% of significance level ($P > 0.05$). The lipid profile of *Spirulina platensis* samples *in nature* and dried showed a polyunsaturated and saturated fatty acids (PUFA/SFA) ratio of 0.88, being the recommendation for the human diet a ratio higher than 0.45. The palmitic and gamma-linolenic acids presented the highest values (32.5% and 20.6%, respectively) of the fatty acids compositions of *Spirulina* fresh and dried product.

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