PHYCOCYANIN CONTENT OF *SPIRULINA PLATENSIS* DRIED IN SPOUTED BED AND THIN LAYER

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ABSTRACT

The aim of this work was to study the drying of Spirulina platensis*, evaluating the final product characteristics about its phycocyanin content and its protein solubility in water. Two drying techniques were used: the spouted bed and the thin layer. For drying in a spouted bed, the cone-cylindrical geometry was chosen, namely type conventional spouted bed (CSB) and jet-spouted bed, with a paste concentration of 5%. The thin-layer drying was performed at temperatures of 50 and 60C, with a load of material of 4 kg/m^2 in the tray. The spouted bed dryer type CSB demonstrated good functionality, not presenting a collapse during the experiments. The solubility in aqueous mean was similar in all the drying techniques used, being the values found around 37%. The largest phycocyanin values were found in the thin-layer temperature of 50C and in the spouted bed type CSB; however, the thin layer was excluded so as not to reach the commercial moisture content.*

PRACTICAL APPLICATIONS

Phycocyanin is the major phycobiliprotein in *Spirulina*. Phycocyanin has significant antioxidant, anti-inflammatory, hepatoprotective and radical scavenging properties. It is used as colorant in food and cosmetics. It was also shown to have therapeutic value (immunomodulating activity and anticancer activity). The drying operation is commonly used to prolong the shelf life of microbial biomasses. Preservation of cyanobacteria is a difficult process, since the cells are small and, moreover, the cultures are usually diluted. Drying of liquids and pastes in spouted beds with inert bodies has been presented as an

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alternative to spray drying in an attempt to obtain high-quality powdered products at a low cost.

INTRODUCTION

Phycocyanin is a photosynthetic pigment of the phycobiliprotein family. Among cyanobacteria, the species of genus *Spirulina* are a rich and inexpensive source of this pigment. It is also a potential therapeutic agent in oxidative stress-induced diseases (Minkova *et al.* 2003).

Drying operation is commonly used to extend the shelf life of microbial biomass, especially if the biomass is the final product. Drying methods have been extensively utilized for biomass drying including spray dryers, cylinders, freeze-dryers and also solar. Freeze-drying method may be considered as one of the references for cyanobacterias because of the minimum alterations on its nutritional, sensorial and physiochemical properties, suggesting that the lyophilized product resembles the fresh biomass (Morist *et al.* 2001).

Drying and storage conditions are factors that may affect the functional properties of the isolate protein as *Spirulina*. Few studies refer to the drying of cyanobacteria, which indicates a need for a better evaluation of preservation methods for this type of raw material.

Sarada *et al.* (1999) relate a loss of approximately 50% of the phycocyanin presented in the biomass when different techniques have been used to dry cyanobacteria *Spirulina*, including the spray and convective dryers.

Spouted bed has been developed to dry large and difficult-to-fluidize, polydisperse particles, which are common characteristics of food products. This dryer has been used successfully to dry various food products (Devahastin *et al.* 2006). Drying of pastes and suspensions in the spouted bed has been shown as an alternative for the drying in spray, providing high-quality and low-cost products (Medeiros *et al.* 2004). Spouted bed dryers with inert bodies have been extensively studied for the drying of bioresources such as foodstuffs and phytopharmaceutical products. Recently, spouted beds have been applied for the drying of medicinal extracts (Shuhama *et al.* 2003; Peixoto *et al.* 2004; Souza and Oliveira 2005).

The prediction of the moisture exchange between air and foodstuffs is very important because it affects the properties and shelf life of the latter. Equilibrium isotherms that relate equilibrium moisture content and water activity at a given temperature and pressure provide a way to describe the hygroscopic properties of foodstuffs (Delgado and Sun 2002).

The exponential model has been widely used to estimate the drying constant of various products with high moisture content, establishing that the

drying rate is proportional to the free water content of the material. Thus, Eq. (1) is an analogous expression to the cooling Newton law, considering that resistance to moisture transport is concentrated inside the material, neglecting the effects of the boundary layer (Chirife 1983).

$$\frac{d\bar{X}}{dt} = -K \times \left(\bar{X} - XE\right) \tag{1}$$

The aims of this work were to characterize the drying of *Spirulina platensis* and to analyze the phycocyanin content and the protein solubility in water of the biomass dried in both the spouted bed and the thin layer techniques.

MATERIALS AND METHODS

Biomass

The microalga *S. platensis* cepa LEB-52 utilized was provided by the Biochemical Engineering Laboratory at Fundação Universidade Federal do Rio Grande by the proposed method of Costa *et al.* (2004). The microalga was cultivated at a pilot-plant in the South of Brazil; it was grown in a Zarrouk medium. *Spirulina* was grown in open raceway ponds of 650 L. Before harvesting, the biomass was filtered and pressed until it had a moisture content of 76% (wet basis, w.b.).

Drying Procedure

Sorption Isotherm. Equilibrium moisture contents of the samples were determined using isotherms obtained at 50 and 60C. The static gravimetric method with sulfuric acid solutions at different concentrations was used. The experimental apparatus consisted of 11 glass bottles, with a height of 7 cm and a diameter of 6 cm. Each bottle was filled until a quarter deft with various concentrations of sulfuric acid solutions (20–70%, p/p) to keep a relative humidity of 5–89% inside the bottles. The samples placed on a support in each bottle were not in contact with the acid solution. For each experiment, 3 g of samples was measured in an electronic balance with a precision of 0.001 g; it was placed into each bottle with a controlled temperature. Measures of the mass values were taken at the 1st, 7th, 10th and 14th days (until a constant weight was reached). Afterward, the moisture content analysis was carried out in order to determine the equilibrium moisture content. Each experiment was accomplished in triplicate.

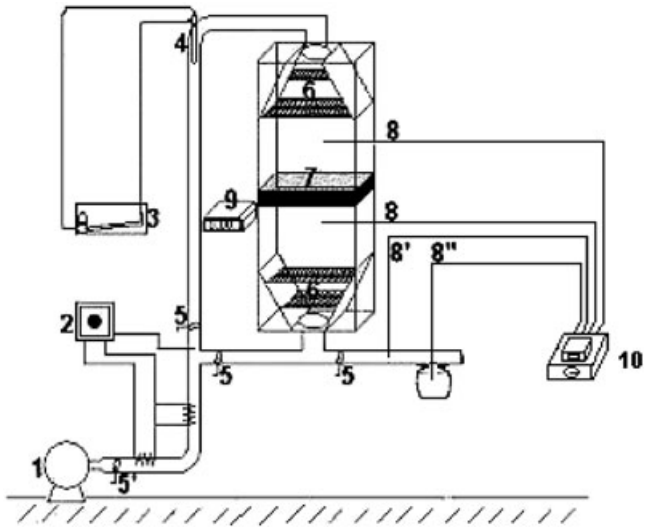

FIG. 1. DISCONTINUOUS DRYERS OF TRAYS WITH PERPENDICULAR AIR FLOW
1, centrifugal fan; 2, temperature controller; 3, manometer; 4, pitot tube; 5, valve of air deviation; 6, air distributor; 7, tray perforated; 8, thermocouples, (′) dry bulb, (″) wet bulb; 9, electronic scale; and 10, milivoltmeter.

The Guggenheim–Anderson–De Boer (GAB) model, Eq. (2), has been usually used to fit the data of food products with satisfactory results. As a consequence of its theoretical origin, its adjustable model parameters have a physical meaning, i.e, X_m is the monolayer moisture content (dry basis, d.b.), C_G and k are constants related to temperature (Mulet *et al*. 2002).

$$X_E = \frac{X_m \times C_G \times k \times RH}{(1 - k \times RH)(1 - k \times RH + C_G \times k \times RH)} \quad (2)$$

Thin-layer Dryer. The drying experiments of *Spirulina* were realized in the thin layer, using the equipment presented in Fig. 1.

The perforated tray was in a rectangular form (0.019 m of width and 0.025 m of length), and the air-drying conditions were temperature of 60C and relative humidity of 10%, and temperature of 50C and relative humidity of 15%. The air velocity for both temperatures was 1.5 m/s.

The biomass was cultivated in tanks, filtered and pressed until it had a moisture content of 76% (w.b.). Drying was realized with the samples in long pellets in a cylindrical form with a diameter of 0.003 m. The sample mass was measured in the electronic balance with 0.01 g of precision; the load of

material used in the tray was 4 kg/m^2. The air-dry bulb temperature was measured at two points, the entrance and the exit of the tray and the wet bulb temperature at the dryer exit, using thermocouples (copper-constantan). Measurements of the sample mass and temperature were taken every 5 min during the drying experiments. The sample was dried until it reached the commercial moisture content (less than 8% w.b.). A moisture content higher than 8% results in the growth of molds and bacteria in the product (Belay 1997).

The drying operation periods were analyzed by the drying curves of dimensionless moisture content ($\bar{X}/X_0$) in function of drying time.

Integrating Eq. (1) from zero (the time in which the material moisture content is X_0) to t (the time in which the material moisture content reaches X) results in Eq. (3), which presents one adjustable parameter K (Chirife 1983; Mujumdar and Menon 1995).

$$\left(\frac{\bar{X} - X_E}{X_0 - X_E}\right) = \exp(-Kt) \tag{3}$$

The proportionality factor K is denominated by the drying constant, being a function of the air velocity, air temperature, relative humidity of air, initial moisture content and thickness of material to be dehydrated.

The drying constant (K) was determined from the curves of dimensionless free moisture content ($[\bar{X} - X_E]/[X_0 - X_E]$) as a function of time (t), using Eq. (3). A nonlinear regression using the quasi-Newton method of software Statistica for Windows 5.0 (Microsoft, Redmond, WA) was performed to obtain the value of K.

Spouted Bed Dryer. Dryers of cone-cylindrical geometry of the types CSB (conventional spouted bed) and JSB (jet-spouted bed) were used. A cell of cone-cylindrical geometry was utilized: a conical basis of glass with an enclosed angle of 60° and a height of 0.15 m for the two dryers. The cylindrical column had a diameter and a height of 0.175 and 0.75 m, respectively, for the CSB dryer, and 0.175 and 0.104 m for the JSB dryer. The CSB drier had a relation between the cell diameter (Dc) and the air inlet diameter (D_i) equal to 6, and that of the JSB drier was equal to 3.5. The bed inert particles were constituted of polyethylene lentils with a diameter 0.003 m, a sphericity of 0.7 and a density of 960 kg/m^3. A mass of 2.5 kg of inert particles was used. The setup of the equipment used in the drying experiment in the spouted bed is shown in Fig. 2:

The system was constituted of a radial blower with a power of 6 kW and a maximum outflow of 0.1 m^3/s. The air was heated in a system composed of three electric resistances each of 800 W. The air velocity of drying was measured in

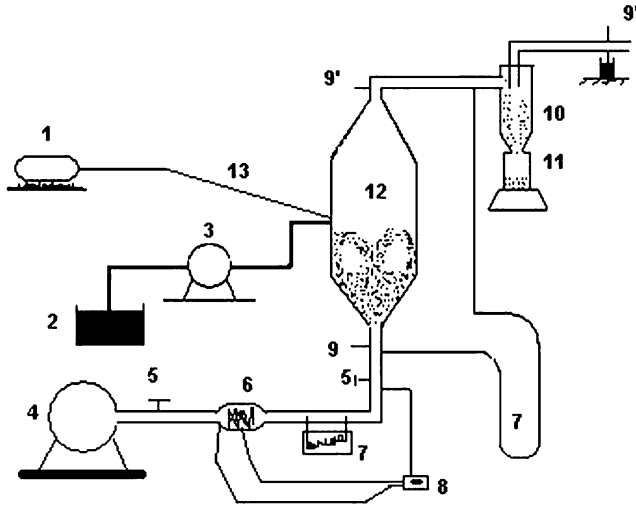

FIG. 2. EQUIPMENT FOR DRYING IN SPOUTED BED
1, compressor; 2, paste reservoir; 3, peristaltic pump; 4, blower; 5, valve; 6, air heater; 7, manometer; 8, temperature sensor; 9, thermocouples, (′) dry bulb, (″) wet bulb; 10, lapple cyclone; 11, final product colleting; 12, drying cell; and 13, atomizer.

an orifice meter and a pressure drop through the stream bed with a U-tube manometer. The temperature readings were carried out in copper-constantan thermocouples. The *Spirulina* solution was fed with a peristaltic pump, and the atomization-type double fluid was done using compressed air with a manometric pressure of 1×10^5 Pa. The dry *Spirulina* was collected in a Lapple cyclone.

Before being fed at the drying cell, *Spirulina* was diluted at a concentration of 5%. The drying conditions in the spouted bed established in preliminary tests were feed rate of paste of 0.4 kg/h·kg of inert, circulation rate of air (v/vjm) of 1.8 and air-drying temperature of 110C ± 3 for CSB and 98C ± 2 for JSB. These experiments extended for 2 h; the first half hour being for the equipment to reach a steady state. In performing the analysis, the samples were collected every 30 min during the time of drying.

Evaluation of Dryer Performance. Dryer performance was evaluated through determination of mass accumulation rate in the bed (*Ac*) and product recovery rate (*R*). The accumulation rate in the bed and the product recovery rate were estimated by mass balance in the dryer using Eqs. (4) and (5):

$$\% \, Ac = \frac{(m_{FB} - m_{IB})\left(1 - \overline{X_F}\right)}{m_I} \times 100 \tag{4}$$

$$\% R = \frac{m_C\left(1 - \overline{X_F}\right)}{m_I} \times 100 \tag{5}$$

For all experiments, the total solid mass introduced into the dryer, m_i; the total mass collected by the cyclone, m_C; the total bed mass at the beginning, m_{IB}, and the end of the process, m_{FB}; and the final moisture content of the material, $\overline{X_F}$, were measured.

Analytical Methodology

Spirulina composition was determined through analytical methods of nitrogen-total, moisture content, ash and lipids, according to the analytical norm of the Association of Official Analytical Chemists (AOAC 1995). The carbohydrate composition was done by difference.

The solubility in water mean was realized by the proposed method of Morr *et al.* (1985) at the initial and final product dehydrated, with some modifications: around 2.5 g of *Spirulina* was added in 50 mL of distillated water, then it was placed in a magnetic agitator for 15 min and was centrifuged at 3,500 rpm for 15 min. After the centrifugation, the tube contents were filtered and the supernatant protein was determined by Micro Kjeldahl by AOAC (1995) with a conversion factor of 6.25. Protein solubility was calculated as the percentage of soluble protein in the supernatant relative to the total protein content in the sample.

The quantitative analysis of phycocyanin was performed by spectrometric method, according to Boussiba and Richmond (1979), at the *in natura* and dried biomass. First, the dried mass of sample was determined, weighing 2 g of sample in the pan and putting it in the oven to dry for 6 h. The calculation of the percent of dried mass was carried out by subtracting the total dried mass of the pan weight, and the result was divided for the wet sample mass. To determine the phycocyanin, 40 mg of *Spirulina* was weighed and 10 mL of phosphate buffer 100 mM was added; this was left in the magnetic agitator to complete dissolution. The samples were stored in a refrigerator at 4C overnight. Then, the samples were mixed and centrifuged at 10C, 3,500 rpm, respectively for 5 min. The absorbency was read at 615 nm using phosphate buffer as blank. The calculation of phycocyanin was performed according to Eq. (6):

$$\% \text{ phycocyanin} = \frac{A_{615} \cdot n_d \cdot 100}{3.36 \cdot (\text{mg sample}) \cdot (\% \text{ dry weight})} \tag{6}$$

where A_{615} is the absorbency in a wave length of 615 nm, n_d is the dilution number, 100 is the representative of 100% and 3.36 is the extinction of coefficient for phycocyanin at 615 nm.

RESULTS AND DISCUSSION

Analysis of the composition was realized for the characterization of *S. platensis* after being pressed and before drying. These results are shown in Table 1.

Drying Characterization in Thin-layer Dryer

The result of the equilibrium moisture content of the sample as a function of the air relative humidity is shown in Fig. 3, for a temperature of 60C, (each experiment was accomplished in triplicate).

The sigmoidal form that is characteristic of food isotherms can be observed in Fig. 3. This behavior was also observed for the temperature of 50C. Table 2 presents the results of the adjustment of experimental data to the GAB model (Eq. 2) for the temperatures of 50 and 60C.

It can be observed in Table 2, by the high t-Student values and for the low standard error, that the GAB equation is appropriate to determine the moisture content of *Spirulina*. Figure 4 presents the residual values for the temperature of 60C, as well as the distribution of the random residues around zero, showing the validity of the statistic used. The same distribution was observed at 50C.

Using the values of GAB, Eq. (3) can be used to determine the equilibrium moisture content of *S. platensis* under the drying test air conditions: for $RH = 15\%$ at 50C, the value found was $X_E = 0.11$ (d.b.); and for $RH = 10\%$ at 60C, the value was $X_E = 0.06$ (d.b.).

TABLE 1.
CENTESIMAL COMPOSITION OF *SPIRULINA* PRESSED

Specification	Composition (%) (wet basis)
Moisture	76.7 ± 0.6
Ash	1.7 ± 0.1
Protein	17.2 ± 1.1
Lipids	2.0 ± 0.1
Carbohydrate*	2.4 ± 0.2

* For difference.

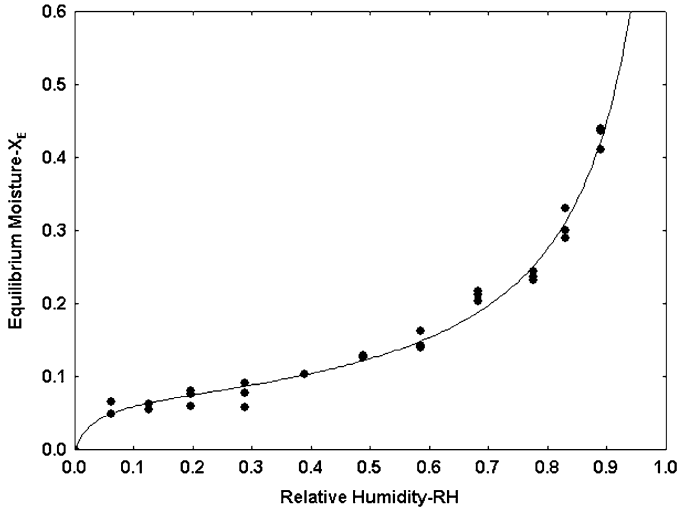

FIG. 3. EQUILIBRIUM ISOTHERM FOR *SPIRULINA PLATENSIS* AT 60C

TABLE 2.
PARAMETERS AND DETERMINATION COEFFICIENT OF GUGGENHEIM–ANDERSON–DE BOER EQUATION AT 60C

Temperature 50C				
Parameters	X_m	C_G	k	R^2
Values	0.11	37.4	0.945	99.45
Standard error	0.002	10.9	0.005	
t-Student	44.4	3.4	197.4	
P level	<0.0001	0.002	<0.0001	
Temperature 60C				
Values	0.068	35.2	0.945	98.65%
Standard error	0.003	20.44	0.008	
t-Student	24.9	1.7	114.6	
P level	<0.0001	0.0962	<0.0001	

The curves describe the behavior of *Spirulina* drying in the thin layer at 50 and 60C for load of solids of 4 kg/m^2, which are shown in Figs. 5 and 6.

Analyzing the dimensionless moisture content curves (d.b.) in function of drying time (Fig. 5), the constant rate period was not verified; it is a characteristic of food with a high protein content (Chirife 1983). In Fig. 5, it is observed that the drying occurred in the first phase of the falling rate period.

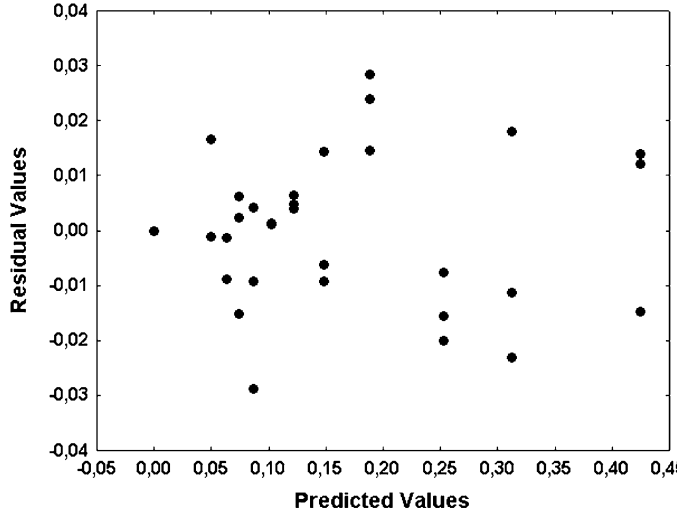

FIG. 4. RESIDUAL VALUES OF GUGGENHEIM–ANDERSON–DE BOER EQUATION FOR *SPIRULINA PLATENSIS* AT 60C

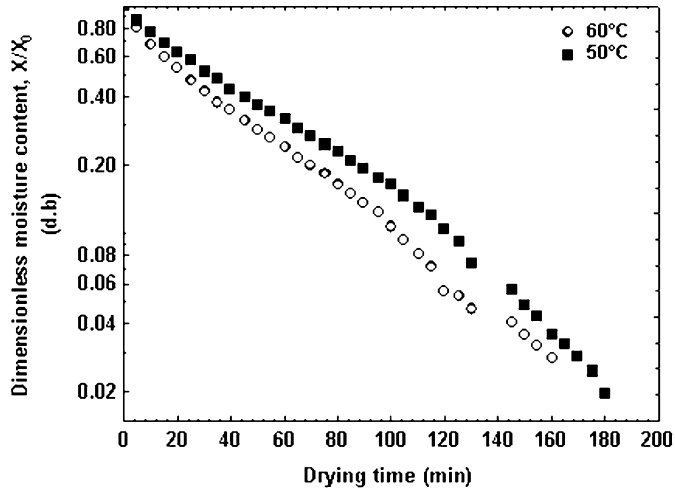

FIG. 5. DRYING CURVE OF MOISTURE CONTENT OF *SPIRULINA PLATENSIS* IN FUNCTION OF TIME, IN SEMI-LOG SCALE, AT 50 AND 60C

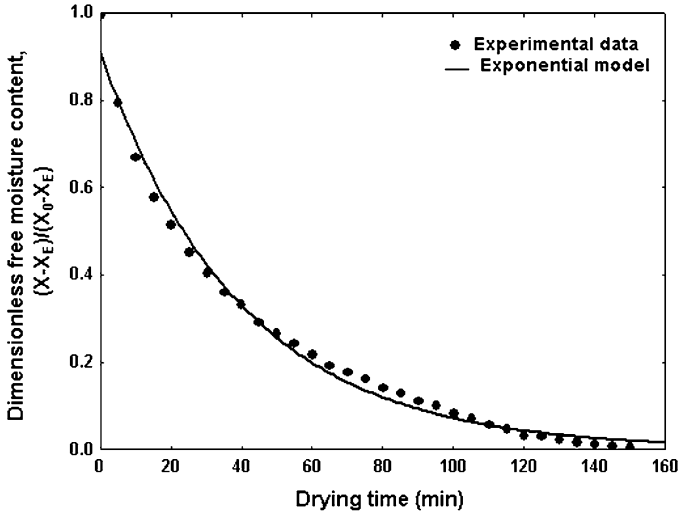

FIG. 6. DIMENSIONLESS FREE MOISTURE CONTENT OF *SPIRULINA PLATENSIS* IN FUNCTION OF TIME AT 60C

TABLE 3.
DRYING CONSTANT AT 50 AND 60C FOR LEWIS' MODEL

Parameters	*K* (1/min)	
	50C	60C
Value	0.022	0.027
Standard error	0.0002	0.0005
t-Student	112	59.3
P level	<0.0001	<0.0001
R^2 (%)	99.56	98.49

The dimensionless free moisture content curve as a function of time, Fig. 6 at 60C, was used to calculate the constant drying (*K*) by Eq. (3).

The drying operation was realized in 2.5 h at 60C to reach a moisture content of 8% (d.b), and in 3 h at 50C to reach a final moisture content of 12% (d.b).

Table 3 presents the values of the parameters of adjustments of experimental data for the Lewis' model (Eq. 3) obtained through the nonlinear regression of the experimental data.

In this table, for the high t-Student values and for the low standard error, it can be observed that Lewis' model is appropriate for the adjustment of the data of the drying of *Spirulina*.

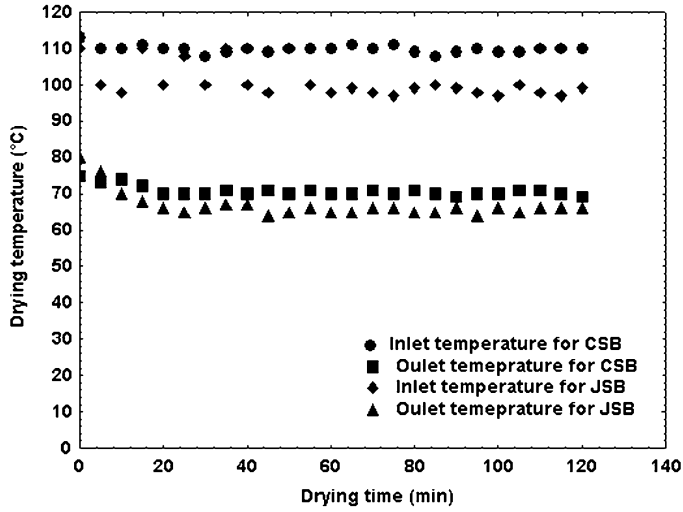

FIG. 7. GRAPH OF TEMPERATURE VERSUS TIME IN DRYING IN THE SPOUTED BED FOR BOTH GEOMETRIES

The Spouted Bed Dryer

Figure 7 presents the profiles of temperature of inlet and outlet in the drying of *Spirulina* in the spouted bed for CSB and JSB geometry.

It can be observed in this figure that both dryers took approximately 15 min to reach the steady state. It can be observed that there is a little variation in the exit temperature, the values of exit temperature being around 70C in both dryers, which guarantees the good characteristics of the dried product.

The powder obtained in drying in the spouted bed in two geometries was 74% passer-by in the mesh 200, with a diameter of particle smaller than 75 μm.

The moisture content of *Spirulina* required for commercial use was reached under drying in the operational conditions in the spouted bed, this value being equal to 6.6 ± 0.2% (w.b.) for CSB and 9.4 ± 0.7 (w.b.) for JSB. The dryer performance evaluated by Eqs. (4) and (5) presented for CSB the accumulation rate of 65% and the product recovery of 28%. The smallest production in the JSB was verified as a result of a larger retention in the bed, because the accumulation rate was 84% and the product recovery was 10%.

Spouted Bed and Thin Layer Drying

The results for protein solubility in water of *Spirulina* and of the phycocyanin content were compared using the Tukey test at the significance level of

TABLE 4.
AVERAGE VALUES FOR PROTEIN SOLUBILITY IN WATER OF *SPIRULINA* FOR THE TWO DRYING TECHNIQUES

Samples	Protein Solubility (%)
Spouted bed dried (CSB)	35.1 ± 4.5 (a)
Spouted bed dried (JSB)	37.9 ± 2.6 (a)
Thin layer dried (50°C)	38.4 ± 3.2 (a)
Thin layer dried (60°C)	37.8 ± 2.5 (a)

The Tukey test with the same letters indicate no significant differences ($p > 0.05$).

5%, with the aid of the program Statistica for Windows 5.0. Averages followed by the same letter did not differ significantly at the level of 95% (smaller significance than 0.05) for the Tukey test.

Solubility. Table 4 presents the results for protein solubility in water of *Spirulina in natura* and after being dried in the spouted bed and in the thin-layer dryer.

Based on Tukey test, for a level of significance of 95%, there was no significant difference between the values of protein solubility in water of *Spirulina* in the spouted bed and the one dried in the thin layer.

Temperature is also a factor that influences protein solubility. In general, protein solubility is increased with temperature between 40 and 50C. When the temperature of the solution is raised high enough for a given time, the protein is denatured. Proteins are denatured by the effect of temperature on the non-covalent bonds involved in stabilization of secondary and tertiary structure for, e.g., hydrogen, hydrophobic and electrostatic bonds. When the secondary and tertiary structures of a protein are unfolded, the hydrophobic groups interact and reduce water binding. Hydrophobic interactions lead to aggregation, followed by coagulation and precipitation. Denaturation decreases protein solubility compared to native protein, and leads to aggregation and difficulty of reversal upon cooling (Laemmli 1970; Mine 1995; Kim 1998; Pelegrine and Gasparetto 2005).

Phycocyanin. Table 5 presents the results obtained for the phycocyanin content of *Spirulina in natura* and after drying; these values have been determined by Eq. (6).

Based on Tukey test, for the significance level of 95%, there was a significant difference between the phycocyanin content of *Spirulina* dried in the spouted bed and dried in the thin-layer dryer, as presented in Table 5.

Table 5 presents that the phycocyanin content was superior in the dry product in the spouted bed and in thin layer to 50C. Shuhama *et al.* (2003)

TABLE 5.
AVERAGE VALUES FOR PHYCOCYANIN CONTENT OF *SPIRULINA* IN NATURA AND DRIED IN TWO DRYING TECHNIQUES

Samples	% phycocyanin
In natura	16.3 ± 0.6a
Spouted bed dried (CSB)	14.7 ± 1.1b
Spouted bed dried (JSB)	10.1 ± 0.8c
Thin layer dried (50C)	15.6 ± 0.5d
Thin layer dried (60C)	12.6 ± 1.1e

The Tukey test with different letters indicates significant differences ($P < 0.05$).

dried annatto (*Bixa orellana,* sp.), a natural pigment, in the spouted bed with temperature of inlet of 60, 80, 100 and 120C; it verified that there was a decrease in the content of the pigment when superior temperatures to 80C were used. Being the same observed in the drying of *Spirulina* in the spouted bed, there was a decrease in the phycocyanin content in the two geometries used. To avoid the degradation of the existent colors in CSB the entrance temperature of 117C was used to guarantee that the exit temperature did not exceed 70C. For JSB, the entrance temperature used was lower (98C) due to the spouted bed JSB to present a vigorous movement of the solid and a contact uniform gas-solid being the exit temperature at 65C. It was observed that the value of the phycocyanin content was superior in the CSB dryer (Table 5); this fact can be explained due to a larger retention presented by JSB and also a smaller production in this bed. The temperature of 50C was excluded by having presented larger humidity.

Morist *et al.* (2001) dried *S. platensis* in a spray dryer and freeze-dryer and obtained, respectively, 1.4 and 4.0% of the phycocyanin content. This was lower than the values reported by Sarada *et al.* (1999) because of the drying methods applied. Also these values were lower when compared with the techniques used in this study (Table 5).

The phycocyanin is obtained by extraction process by solvent and subsequent purification, therefore the drying operation is important in the process to guarantee that the dry product maintains a phycocyanin content. Above 60C, there is a degradation of phycocyanin and an increase of the Maillard reactions.

CONCLUSIONS

For thin-layer drying of samples, *Spirulina* in pressed pellets form ($X_0 = 76\%$ w.b.) at 50 and 60C, only the existence of the falling rate period was verified.

The spouted bed with cone-cylindrical geometry presented a stable behavior during the period of accomplishment of the experiments of drying of *S. platensis*. The operational conditions presented a good operation in the type CSB. The spouted bed JSB presented a larger retention in the bed and smaller product recovery.

The solubility in aqueous mean was similar in all the drying techniques used, the values being found around 37%. The largest phycocyanin values were found in the thin layer at a temperature of 50C and in the geometry CSB; however, the thin layer at 50C was excluded. Therefore, the commercial moisture content was not reached in the final drying in this temperature.

NOMENCLATURE

A_{615}	absorbency at 615 nm	dimensionless
C_G	constant of Eq. (2)	dimensionless
RH	relative humidity	dimensionless
K	drying constant	1/min
k	constant of Eq. (2)	dimensionless
t	time	min
$\overline{X}$	average moisture content	kg/kg_{ds}
X_E	equilibrium moisture content	kg/kg_{ds}
X_0	initial moisture content	kg/kg_{ds}
$\overline{X_F}$	final moisture content	kg/kg_{ds}
X_m	monolayer moisture content	kg/kg_{ds}

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