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# SPIRULINA BIOMASS TREATMENT. PART II COMPARISON OF *SPIRULINA* DRYING PROCESSES ON THE BIOCHEMICAL QUALITIES: CRITERIA OF PROTEINS AND TOTAL SUGARS CONTENTS

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# 1. Problem definition

In this report the drying processes are compared according to their capacities to preserve the protein content and sugars availability. These contents are measured before and after drying by several processes: convective drying, oven-drying, infrared drying, spray drying (or atomisation) and freeze-drying. These processes allow obtaining various presentations, forms and colour of dry *Spirulina*. The bicinchoninic acid (BCA) method and the Herbert method are used respectively for the available protein and total sugars contents determination. [1]. Total sugars are analyzed by the Herbert method [2].

# 2. Materials and Methods

## 2.1. Spirulina

The *Spirulina* culture is grown in a batch reactor of 30 litres under a constant light intensity of 200 W/m$^2$ (see Figure 1). A simplified Zarrouck medium [3] without the A4 and A5 solutions was used. The *Spirulina* was filtered through a 20 µm filter and then washed with distilled water.

A sample of fresh *Spirulina* was frozen which allows the simultaneous biochemical analysis of the dry biomass and corresponding thawed fresh biomass. The moisture content is measured before and after drying. The moisture content of fresh *Spirulina* is taken into account for the biochemical analysis The dry based moisture content X is expressed in kg water per kg dry matter. The protein and total sugars contents are expressed in protein or sugars mass per mass of dry biomass in percentage.

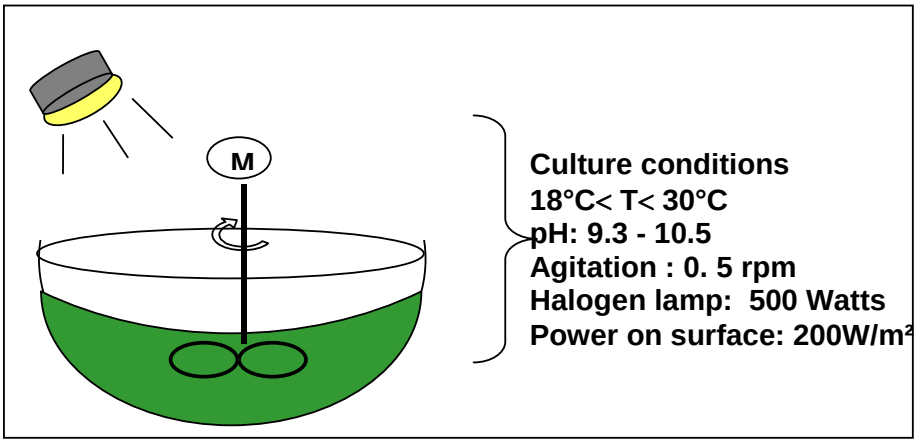


**Figure 1. Discontinuous reactor for Spirulina culture.**

Due to the variation of the exopolysaccharides content (and consequently the proteins percentage) of the fresh *Spirulina*, a new reactor is being building (air lift reactor) in the

laboratory. Its utile volume is 40 L, the light is provided 20 neon lamps (5 are sun spectral light). The agitation is obtained by air lift, at the lower middle part of the reactor. The pH is regulated using a $H_2SO_4$ solution and the reactor temperature is controlled by means of a coil heat exchanger with water provided by a temperature controlled external water bath. Samples can be obtained with a peristaltic pump, what allows the injection of culture medium.

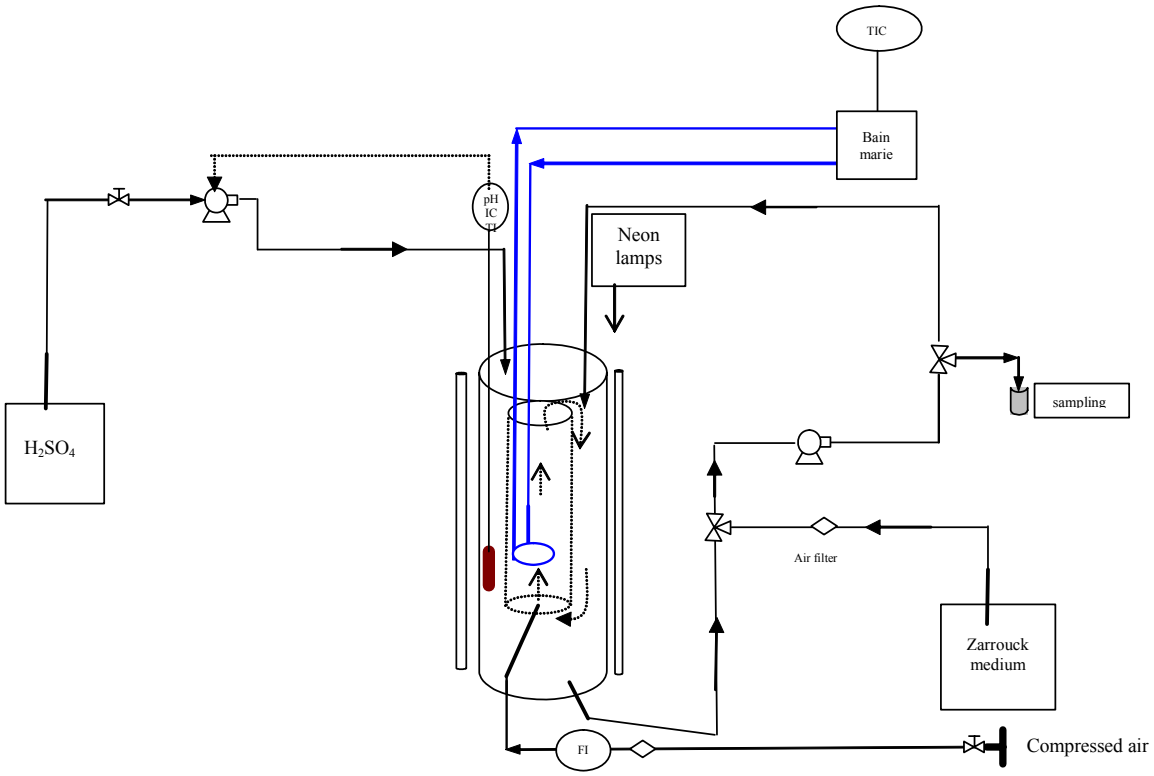


**Figure 2. Air lift photobioreactor for Spirulina.**

In this study, *Spirulina* has been grown and took always from the hemispherical reactor. These new reactor allows to get a better quality of *Spirulina* for further experiences.

## 2.2.Drying processes

### *2.2.1. Oven drying and convective drying:*

Oven-drying is used to study the influence of the drying air temperature on the proteins and sugars contents. The *Spirulina* is spread out on a pan. As soon as the weight is constant, the product is removed to avoid an additional degradation. The average drying time observed is 3h.

Convective-drying is presently used in the small-scale farm of *Spirulina*. Fresh *Spirulina* is extruded and convective dried with hot air; that allows to obtain small cylinders or pellets, they can be crunched in powder or simply added to food without change. The drying experiments are carried out in a closed loop dryer. The *Spirulina* is dried in cylinders or thin layer posed on a plan support, licked by air flow. The air temperature and velocity are fixed. The relative humidity of air is constant; the volume of air is very significant in front of the quantity of evaporated water, which makes it possible to preserve the relative humidity and the constant temperature. The average drying time observed is 3h. The moisture contents initial samples are determined in the oven at 110°C to correspond to the standard temperature to measure the dry mass of biological products (or 60°C at the beginning of the work, but no difference in the corresponding dry mass between the both temperature for the same sample).

### *2.2.2. Infra-red drying:*

This type of drying allows spreading out the fresh *Spirulina* in the form of thin layer or cylinders. With the dryer of the laboratory, the drying temperature is fixed between 40°C and 60°C. This temperature is controlled by the infrared radiation power from 9 lamps of quartz with tungsten filaments of 1kW each one. The lamps are placed at distance of 261 mm from the sample. The drying time is between 40 min and 2 hours according to geometrical characteristics, to the spreading out and the initial water content of the samples.

### *2.2.3. Spray drying:*

A mini spray dryer BUCCHI model B-191 was used. The final product is a powder; the obtained particles diameter depends on the type of the pulverization module, its number of revolutions, the air temperature, and feed rate, the initial concentration of the solution, the total pressure and the flow of the gas air of pulverization. The used drying air temperatures are130 and 150°C. The moisture contents are determined before and after drying.

### *2.2.4. Freeze drying:*

A freeze-dryer USIFROID model SMH45 is used. The *Spirulina* is filtrated and rinsed out with water. The sample must be porous; to ensure a better thermal transfer during the freezing phase and during the sublimation, the vapor transfer under vacuum is facilitated when the pores are large enough. Then the fresh biomass is diluted to a concentration between 0.02 and 0.05 g dry spi /ml (20 kg water/kg dry matter). This optimum concentration was deduced from studies on other products (vaccines) at the laboratory. In the case of *Spirulina*, it could be optimized. The product is dried in flask of 1 ml. The experiment time varies from 24 to 48h. The optimal parameters for the *Spirulina* freeze-drying are not yet known. Some tests were carried out with parameters given for other products. The vitreous transition temperature (Tg) measurement that correspond to the maximum cryo-concentrated phase from the *Spirulina*,

was measured by Differential Scanning Calorimeter (DSC) at the laboratory. For a solution *Spirulina* with 5 kgw/kgdm, the vitreous transition is - 30°C (see figure 3).

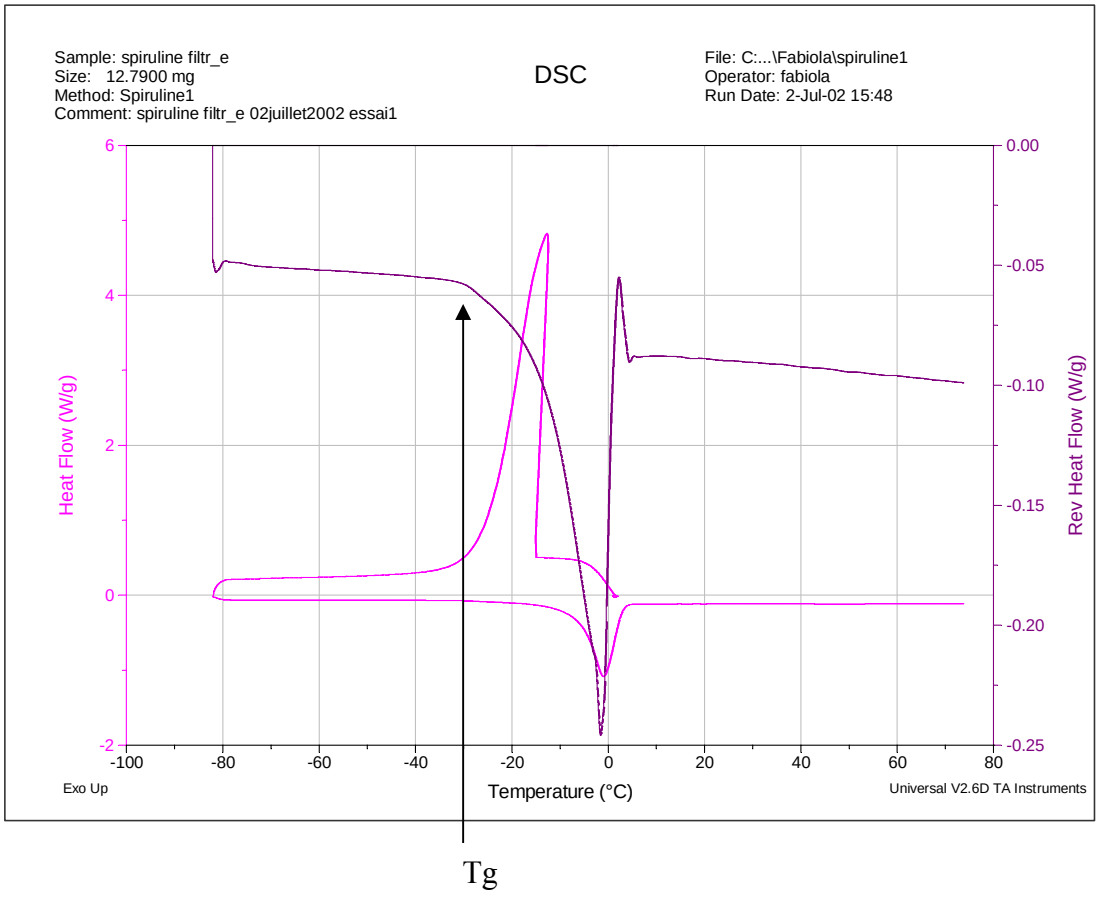


**Figure 3.Thermogram of Spirulina obtained by Differential Scanning Calorimeter.**

The sample freezing step was carried out at -45°C during 4 hours. The ice sublimation phase, called primary desiccation, is carried out by vacuum at 8 Pa and -20°C during 18 h. The second phase or secondary desiccation is carried out on 20°C during 6.5 hours by 1.5 Pa. The moisture content is determined at the beginning and the end of drying. The obtained product is a powder. For a use of freeze-drying in spatial station, it should be necessary to optimize the initial moisture content of *Spirulina* to minimize the total need of energy of drying, to identify the optimum temperatures and freezing rates and the temperatures and time of primary and secondary desiccation to reduce the energy consumption and optimize quality.

## 2.3.Protein determination by the bicinchoninic acid (BCA) method

### *2.3.1. Principle:*

Smith et al. [1] proposed the analysis method where proteins reduce cupric ions to cuprous ions under alkaline conditions. The cuprous ion complexes with apple-greenish BCA reagent to form a purplish colour. The intensity of the formed colour is proportional to protein concentration and determine by reading the absorbance at 562 nm.

$$\text{Proteins} + Cu^{2+} \xrightarrow[OH^- ; H_2O : \text{alkaline medium}]{\text{BCA}} \text{Complexes purplish colour, } \lambda = 562 \text{ nm.}$$

### *2.3.2. Optimization of extraction methods for the determination of proteins:*

The proteins of the *Spirulina* are contained in the cells and the cells constitute a long filament. It is necessary to be able to destroy the cells membrane so that the contents and thus the proteins go in solution. The experiments showed that the extraction of proteins is more difficult to realize on fresh *Spirulina* than on the dried one. Different methods of mechanical and physical extraction for the proteins were assayed to destroy the membrane of the cells using either nitrogen liquid immersion, shear stress by means of high speed homogenization (Ultraturrax) and the ultrasonic treatment. The sonification method (ultrasounds) was the finally selected method because it allows to obtain the highest proteins release. Different experiments were carried out to observe the influence of the sonification power, the sonification time and the duration between the reaction and the reading time of the optical density at the spectrometer (Figures 4, 5 and 6).

Due to the important deviation obtained between analysis of different aliquots of the same samples from convective and infrared drying, some measurements were carried out after crushing the *Spirulina* in a mortar. In this case dried *Spirulina* was hand crushed with a pestle during 5 min. From this homogenized material 10 mg was used for the protein analysis. The results are compared.

### *2.3.3. The dry Spirulina as reference:*

The biochemical analysis are expressed per unit mass of dry *Spirulina*. The initial moisture content $X_i$ is taken into account for the biochemical analysis on fresh *Spirulina*.

X is measured by oven drying with around 2 g of fresh biomass at 110°C until constant weight that often takes around 24 hours. The initial moisture content X is equal to :

$$X = \frac{m_{initial} - m_{final}}{m_{final}}$$

Then , $X = \frac{m_{water}}{m_{dry\ \ matter}}$, in kg water per kg dry *Spirulina*

Then , $m_{dry\ matter} = \frac{m_{fresh\ \ matter}}{X+1}$

The protein content $C_i$ is measured from a sample of fresh *Spirulina* and reported to the dry mass that is content in these fresh biomass.

C is in mg proteins per mg dry *Spirulina* in the dried sample.

$C_i$ is in mg proteins per mg dry *Spirulina* contained in the fresh *Spirulina*.

$$\frac{C}{C_i} = \frac{\text{concentration in protein of the dried sample}}{\text{concentrataion in protein of the fresh sample}}$$

expressed in

$$\frac{\text{mg remaining protein par mg dry spirulina after drying}}{\text{mg protein par mg dry spirulina contained in the fresh sample}}$$

then ,

$$\frac{C}{C_i} \text{ is in } \frac{\text{mg remaining protein after drying}}{\text{mg protein before drying}}$$

### *2.3.4. Experimental protocol:*

After filtration and rinsing the *Spirulina*, around 10 mg of with one studied drying process *Spirulina* are taken or 40 mg of fresh product that contained around 10 mg dry *Spirulina*. The exact weight is measured. This cellular mass is suspended in 10 ml SDS 1%. This mixture is submitted to ultrasonic treatment (Ultrasonic processor model 600 Watts) with a power of 104 Watts during 1 minute (90% from the maximal power that the ultrasonic can delivered to the solution), the small flask is maintained simultaneously in a full with water and ice beaker. This power and sonification time were determined from experiments. The SDS (Sodium Dodecyl Sulfate salt) allows to solubilise extracted proteins. The sample is diluted to 1/ 4 = 50 µl of the sample + 150 µl distilled water to locate the results in the standard curve established from 0 to 250 µg protein/l. A 2 ml aliquot of the BCA work solution is added to 0.1 ml of the diluted solution of *Spirulina*. After homogenisation, the tubes are allowed to rest during 30 minutes at 60 °C. Then, the tubes are cooled in a bath; the optical density is read at 562 nm.

Protein concentration in the samples is estimated based on the comparison with the protein serum bovine albumin (BSA) standard curve, figure 4. The relation between the absorbance and the concentration remains linear in the used range.

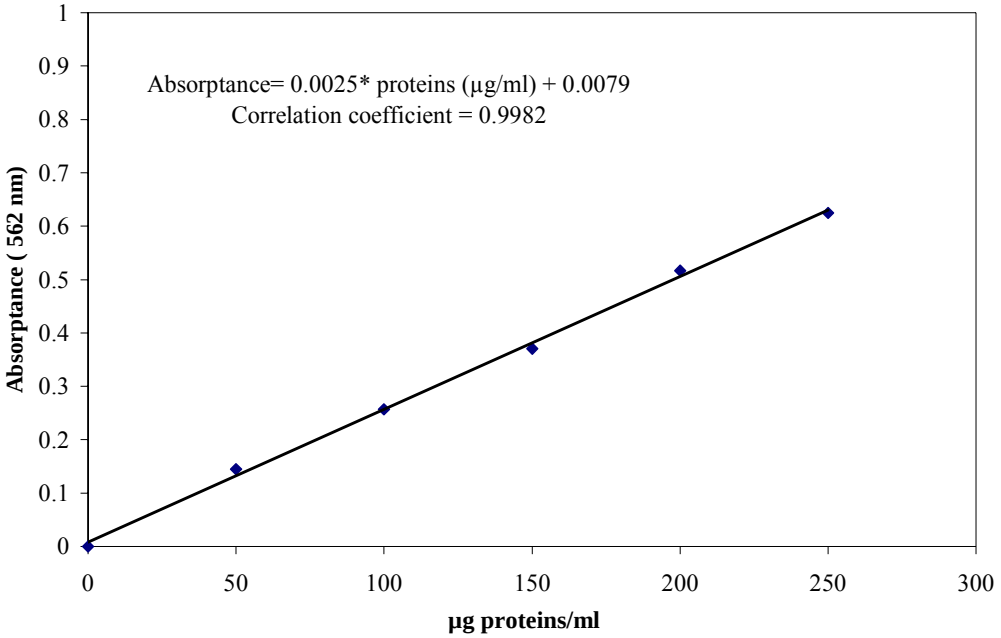


**Figure 4. Calibration curve with Bovine Serum Albumin for the protein analysis.**

## 2.4. Total sugars determination method

### *2.4.1. Principle:*

The colorimetric phenol method, described by Herbert et al. [2] allows determining the total sugars concentration in the *Spirulina* samples. The hydrolysis of polysaccharides into monosaccharide units is carried out by heating in acidic medium. Monosaccharides are then reorganized to give either furfural (in the case of pentoses) or hydroxyméthylfurfural (in the case of hexoses). These compounds react with phenol to form a coloured compound whose absorbance can be measured at 480 nm.

### *2.4.2. Experimental protocol:*

A sample of 40 mg of fresh *Spirulina* or 10 mg of dry *Spirulina* is weighted and resuspended in 10 ml distilled water. 0.5 ml of phenol is added to 0.5 ml of the sample. Then 2.5 ml of

sulphuric acid are added and mixed. After waiting 10 minutes, the tubes are cooled during 15 minutes at 25°C. The reading of the optical density at 480 nm allows the measure of the quantity of sugar. The standard range curve, figure 5, is obtained with a glucose solution from 0 to 100 mg glucose/l.

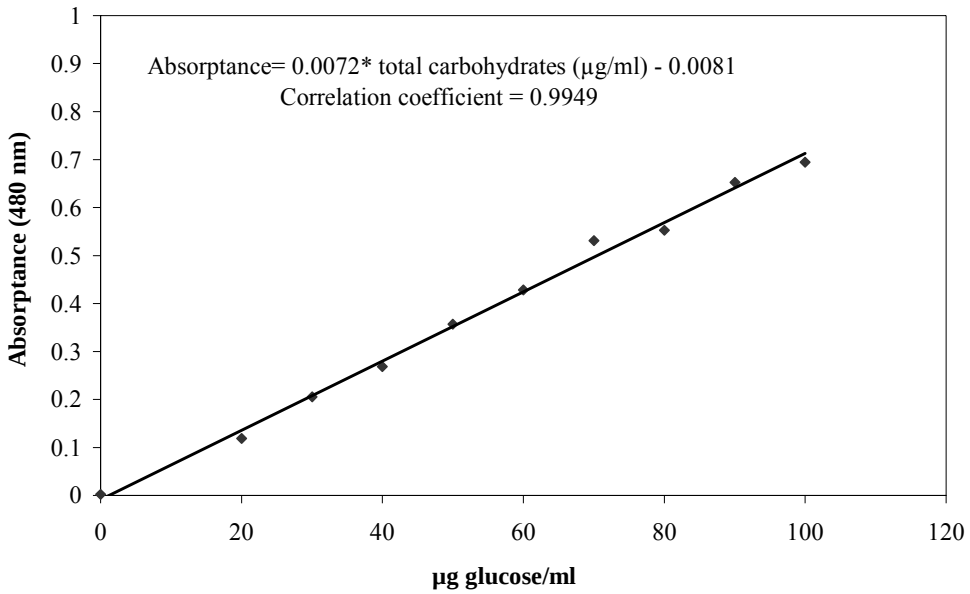


Figure 5 : calibration curve for the sugar analysis, standard with glucose.

## 2.5. Treatment of the samples

The harvested biomass is dried by all the studied processes. To allow a comparison, part of the fresh *Spirulina* is frozen to be thawed for the biochemical analysis of the corresponding dried samples. The culture in batch is not controlled, then the proteins content of fresh *Spirulina* is very variable from one fresh sample to another taken at different culture time. Because of the weak precision of the proteins analysis in complex medium, the analyses are made by two manipulators. It is the tendency of the variation of the concentration which was taken into account with the variation of C/Ci of proteins and sugars.

# 3. Results and discussion

In order to compare the different methods *Spirulina* samples were submitted to the drying procedures assayed. The drying conditions used are summarized in table 1.

The initial moisture content Xi is measured from a wet sample before drying for each experiment, as explained on §2.3.3. The thickness is measured with a micrometer.

**Table 1: Drying conditions used for the different used drying methods.**

| Drying process | Moisture content or initial concentration | Form of product initial | Form of product final | Drying temperature | Others fixed conditions | Drying time |
|---|---|---|---|---|---|---|
| Oven-drying | 4 <Xi<8 kgw/kgdm | Fresh paste | Thin layer | 40°C<br>60°C<br>70°C | | Around<br>3 h |
| Convective drying | 4 <Xi<8 kgw/kgdm<br>(kg of water/kg dry matter) | →Thin layer<br>30 x 40 mm.<br>Thickness :<br>1-1.5 mm | →Thin layer :<br>25 x 30 mm<br>Thickness :<br><1 mm | 40°C<br>50°C | Air velocity :<br>0.15 m/s<br>4 < HR < 7% | Around<br>3 h |
| | | →Cylinders<br>Φ = 2-3 mm | →Thin layer:<br>Thick.<1mm | 60°C | | |
| Infrared drying | 4 <Xi<8 kgw/kgdm | →Thin layer:<br>30 x 40 mm<br>Thickness =<br>1-1.5 mm | →Thin layer :<br>25 x 30 mm<br>Thick.<1mm | 40°C<br>50°C | | 40min - 2 h |
| | | →Cylinders<br>Φ = 2-3 mm | →Thin layer<br>Thick.<1mm | 60°C<br>70°C | | |
| Spray drying | 0.01 g dry Spi./ ml w<br>Xi = 100 kgw/kgdm | Liquid suspension | Powder | 130°C<br>150°C | Feed rate<br>0.09 l/h | few seconds |
| Freeze drying | 0.02 – 0.05 gr dry *Spirulina* / ml water<br>Xi= 20 kgw/kgdm | Liquid suspension | Powder | -20 °C<br>then<br>+20°C | Freezing:-45°C<br>1st desiccation:<br>-20°C ; 8 Pa.<br>2nd desiccation:<br>20°C; 1.5 Pa. | -> 4,2 h<br>-> 18 h<br>-> 6,5 h<br>Σ= 28,7 h |

## 3.1. Optimization of extraction methods for the determination of proteins:

As mentioned before the ultrasonic treatment is the method that allows the highest proteins content and a better reproducibility. The sonification power and time were studied to find the optimal values of the sonifier parameters with one type of *Spirulina* : the commercial spray dried *Spirulina* in a homogeneous composition in the bottle due to the same origin and fine powder presentation.

The figure 6 shows the influence of the sonification time for a constant power of 120 Watts and the figure 7 show the influence of the power for a sonification time of 1min. The effect of the length of the time period between the cooling of the samples and the reading of the optical density can be observed in figure 8.

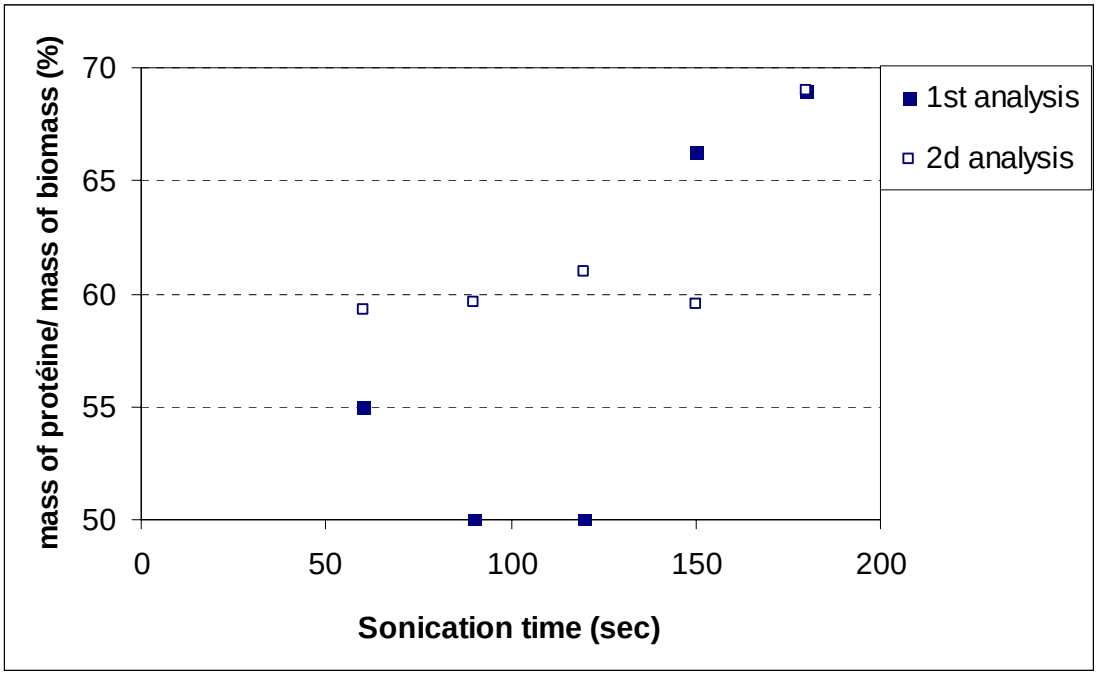


**Figure 6. Influence of sonification time on the proteins content of a sample of commercial Spirulina, power = 120Watts.**

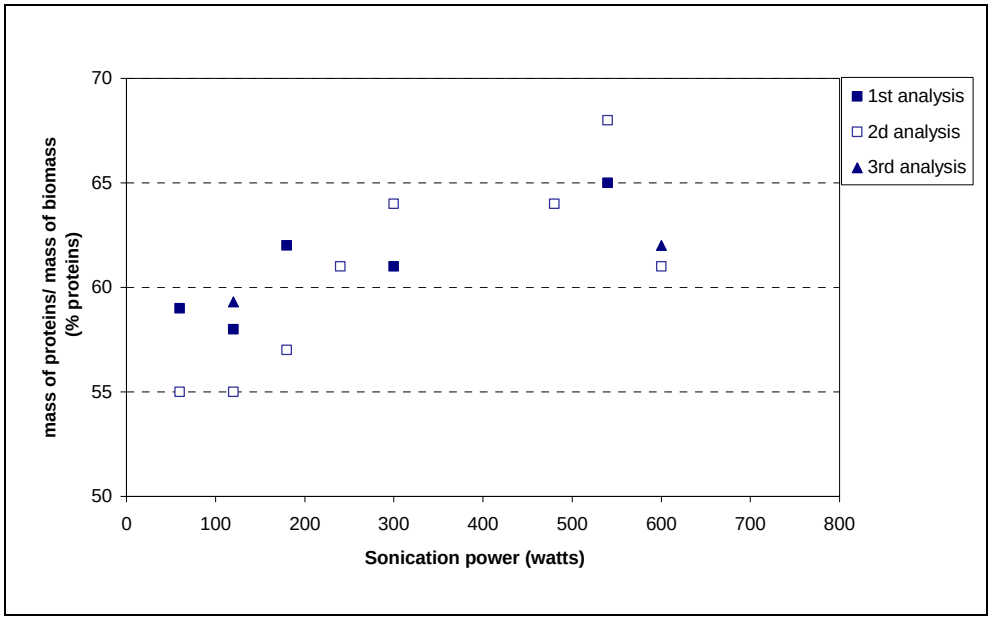


**Figure 7. Influence of sonification power on the proteins content of a sample of commercial *Spirulina*, the power is applied during 1 mn and indicated as consumed power by the sonifier.**

On figure 4, for the 1st analysis series of the spray dried *Spirulina*, the protein content is constant up to 125 sec of sonification time and then increased. For the second analysis, the protein content is 50 or 55% up to 125 sec (these low deviation of 9% is in the total precision of 13%, see 3.2.1), that means it is quite constant, then the protein content increase after 150sec sonification. Then , the analysis were done during 60 sec sonification.

For a duration of sonification of 1mn, the optimal power is between 300 and 540 Watts. Some experiments show that the protein contents measured are the same after 120 Watts-3 min. or 540 Watts - 1min, this allows to limits the sonification time to one minute for a power of 540 Watts. At consumed sonification powers above 540 Watts, it appears a great quantity of foam above the solution which results in a loss of proteins due to a loss of the sample from the bottle. The foam comes from : - the SDS, without consequence; - a partial and light denaturation of some proteins, but from some biochemical researcher, it has no effect on the quantitative analysis, because quite no effect on the peptide link 540 Watts is chosen for the others analysis.

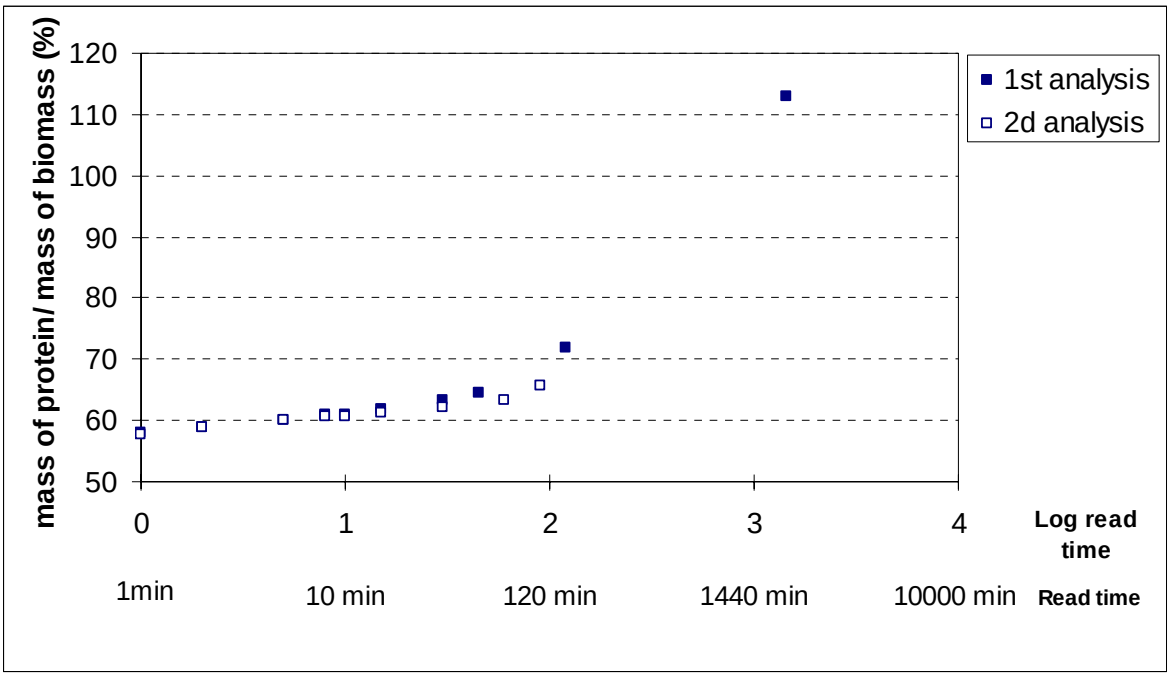


**Figure 8. Influence of resting time before absorbance reading on the protein content of a sample of commercial Spirulina.**

The duration of the resting time before absorbance reading on the samples shows a increasing of the absorbance after 10 minutes and strongly after 1440 mn. Therefore it was decided to perform the measurements at times below 10min because of the exponential increment of the absorbance measure if a longer resting time is used. That avoid to get a dependency between the reading time and the protein content. These identification of the optimal conditions are made on *Spirulina* to adapt the protocol (establish on BSA) to the *Spirulina*.

From Smith et al (Smith et al, 1985), the proteins analysis with BCA method after the incubation timing by 37°C/30 min or ambient temperature during 2 h, show that the absorbance (read proteins content) is always increasing with the incubation time.

## 3.2.Influence of drying method on the protein content

### *3.2.1. Precision*

The influence of the drying process are directly measured on the obtained dried *Spirulina* and not on a reference protein such as BSA or another albumin. The analysis protocol were used several times on a single sample : Two samples, one convective dried, one freeze dried, were

several time analysed. The results are in table 2. The standard deviation are ± 6.92 and ± 6.75%, then the possible deviation is around 13%.

**Table 2 : Analysis of infrared dried Spirulina at 60°C, 8 times, and freeze dried Spirulina, 10 times, with the same protocol.**

| Repeatability | Infrared 60°C 8 analysis | Freeze drying 10 analysis |
|---|---|---|
| Proteins content in % | 61.66 | 72.63 |
| | 65.00 | 82.13 |
| | 55.63 | 66.95 |
| | 53.78 | 77.51 |
| | 62.98 | 78.53 |
| | 68.14 | 72.70 |
| | 74.90 | 76.30 |
| | 68.19 | 90.48 |
| | | 84.03 |
| | | 82.50 |
| Average protein content in % | 63.78 | 78.37 |
| Standard deviation in % | 6.92 | 6.75 |

The protein analysis has been repeated with the same protocol on a commercial *Spirulina* (spray dried *Spirulina* from Earthrise), 14 times, with another research worker, the average value was 75.6% with a standard deviation of ± 9.7% (that means a maximal deviation of 19.4%). The total sugars has been analysed, by the same person, with these *Spirulina*, the average value 14.2% with a weak standard deviation of ±1.6%.

### *3.2.2. Oven-drying and convective drying*

*Effect of the air temperature*

The effect of different air temperatures in the oven-drier on the protein content of the samples is shown in the figure 9. For these tests, for one curve on figure 9, one sample of *Spirulina* is harvested, filtrated and rinsed. Then, 3 aliquots are taken from these fresh *Spirulina* and dried simultaneously in three oven dryer where the air temperature are fixed (40, 60 or 70°C). These operation is repeat 5 time. Protein content is measured in the fresh sample and in each dried sample. Then , on figure 9, one point correspond to one protein analysis.

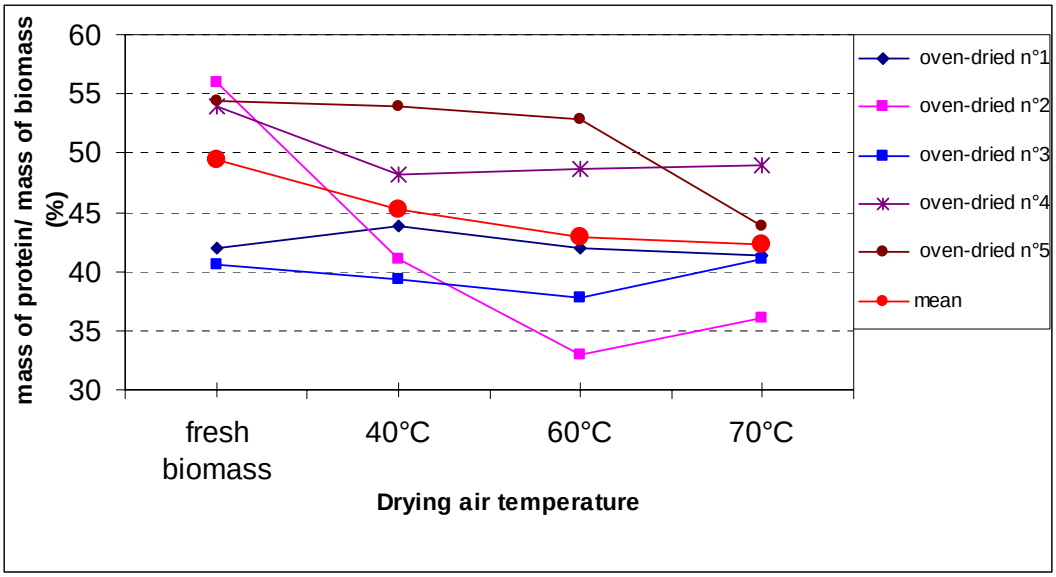


**Figure 9. Influence of air temperatures on the protein content for 5 samples dried in the oven-dryer.**

As can be seen in figure 9, the protein content values are scattered according to the experiments of drying, which correspond to different samples of *Spirulina*, taken at different days from the culture and thus with a variable initial proteins contents. Most of protein analysis show the tendency of a decreasing of the proteins contents in function of the temperature up to 60°C. Between 60°C and 70°C, three analysis show a decreasing of proteins and three show a constant or increasing of proteins contents. But the maximal deviation of 12% (experiments n°2 and 3) is in the minimal precision obtained in the standard deviation measurement (13%) § 3.2.1. Dispersion observed can come from two reasons : one come from the variation of the fresh *Spirulina* composition (exp 1 to 5), the second comes from the precision measurement of proteins. For this reason the results will be expressed as the ratio of C prot.-concentration after treat./ C prot concentration of the fresh *Spirulina*, on figure 10.

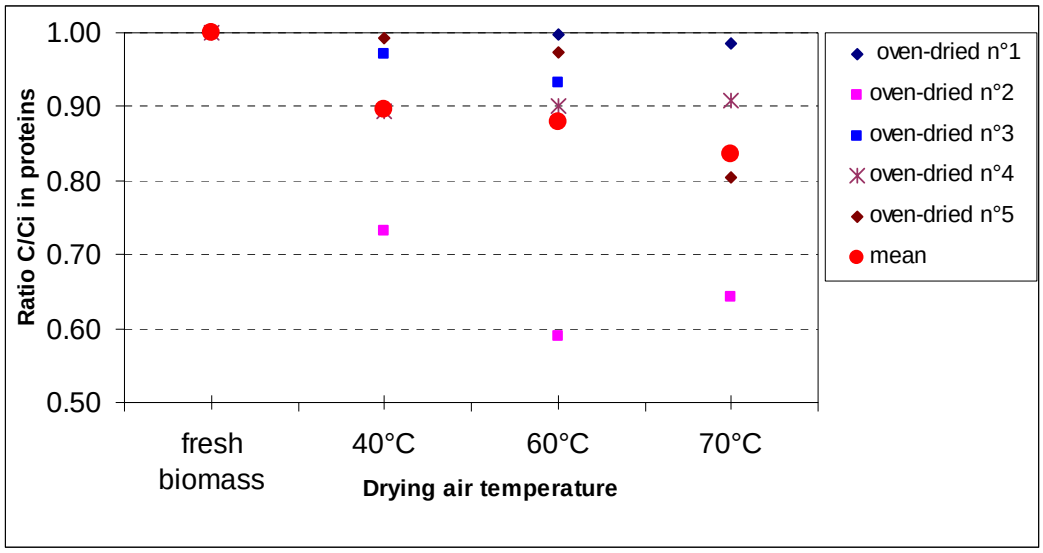


**Figure 10. Ratio "protein content after drying/ protein content initial" for 5 samples in function of the drying air temperature in the oven -dryer.**

On figure 10, the proteins loss is slightly proportional to the temperature between 40°C and 70°C respectively from 10 to 20%. The evolution in function of the temperature is the same as in figure 9 but allows a better comparison.

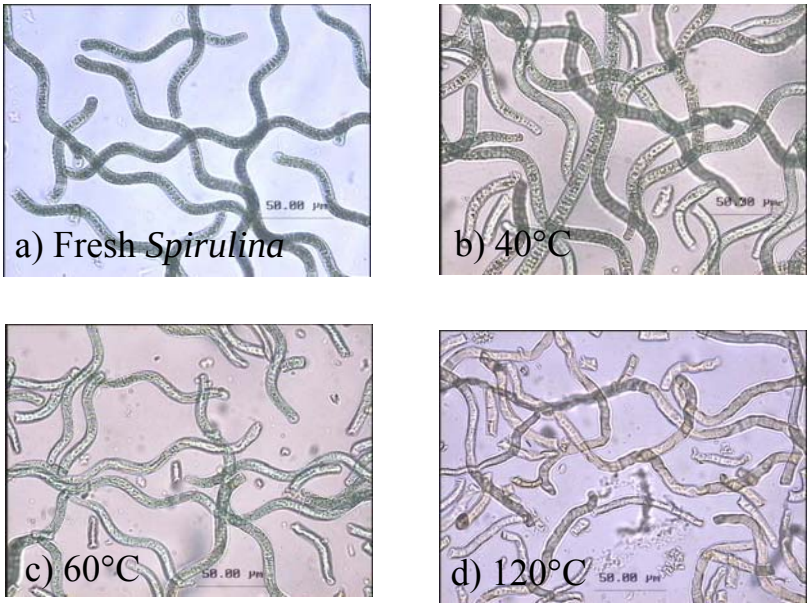


**Figure 11. Photomicrographs of filaments of fresh and oven-dried Spirulina at different air drying temperatures (enlargement 50X).**

Figure 11 shows the filament obtained after oven drying at different temperatures, after rehydration with water. At 40°C no damage is observed. Damage on the filament increased for higher temperatures from 60°C to 120°C. The damage appears especially on the edge of the filament. These can be probably due to the composition cell walls of *Spirulina*, with lipopolysaccharide and peptidoglycan layers *(Drews and Weckesser, 1982)* sensible to heat. The border of the filament becomes smooth. The brightness of the filament decreased as a function of the air drying temperature.

*Influence of crushing the Spirulina*

On the fig 13, the same experiments are carried out as on figure 10 at the temperature of 40, 50 and 60°C. The samples were crushed during 5 min. with a mortar and a pestle before the protein measurement. Although crushing brings energy to the product, the measurement of the temperature under the aluminium capsule shows, on figure 12 that the increasing of the temperature during the crushing is low.

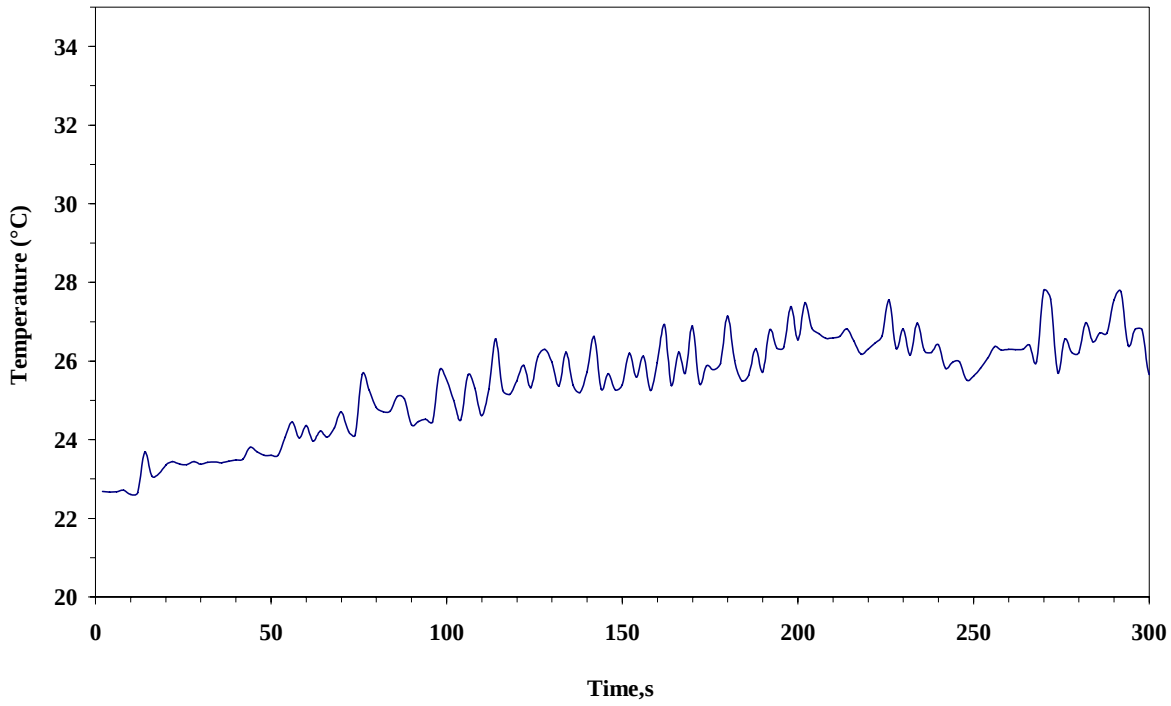


**Figure 12 : Temperature evolution during the crushing of *Spirulina,* in a mortar.**

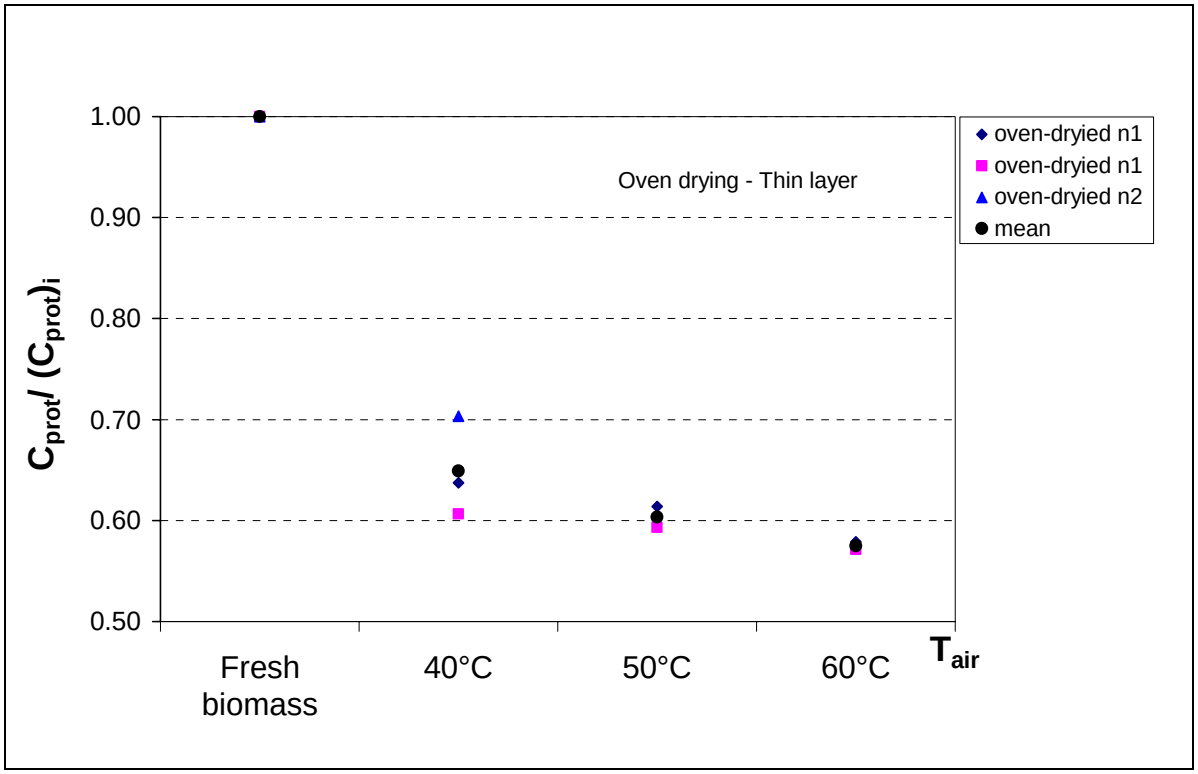


**Figure 13 : Protein losses after oven drying. The samples were crushed before proteins analysis.**

The analysis show that the protein content is more reproductive, but the protein content is lower than the first method: the deviation of the remaining protein (in ratio to the fresh content) vary from 60% to 100% without crushing and from 60 to 70% after long crushing. Two interpretations can explain this difference: if the results without crushing are more real, the protein can be damaged by a long crush, if the results with the crushing are right, that means that the method without crushing occurs more interference for the absorbency reading, or the real protein content is really low and the crush allows a better homogenisation of the samples.

In spite of this difference, between 40 and 60°C, both results show that the higher the temperature, higher the protein content.

Nevertheless, these results were carried out after all the other analysis. That mean that the next results on figure 14 to 21 are obtained without crushing the dry *Spirulina*. For these measure, *Spirulina* cells are crushed only with ultra-sound treatment as presented in the experimental protocol for the protein analysis on 2.3.4. Further analysis will be carried out to confirm or not the observed tendency of the influence of the drying processes on *Spirulina* biochemical properties, with crushing the dry *Spirulina* in the protocol of proteins and sugars analysis.

*Influence of the form of spreading out the Spirulina*

Figure 14 shows a very high dispersion, up to 50%, of the results of the analyses for the same sample, if all the results are taken into account. Dispersion depends much on the type of drying, as the results by freeze-drying and by atomisation (following figures) show a better reproducibility for the same sample. This is probably due to homogeneity of the sample to dry. A high temperature involves phenomena of crusting. A possible localisation of exopolysaccharides on the dry sample can be observed by drying in thin layer, with the appearance of irregularly scattered yellow spots, which seems absent for the other processes. It would be interesting to try to obtain the profile of a protein concentration in the thickness of the product final (thin layer or cylinders).

On the same figure, the protein loss is closed for the samples dried with the drying oven and the convective drier. It appears to exist a small influence of the form of spreading out, but it is not clearly marked. With 40°C, at convective and infra red drying (see figure 14 and 15) the loss is slightly larger for cylinders samples than for the thin layer ones. But these results have to be checked by other experiments.

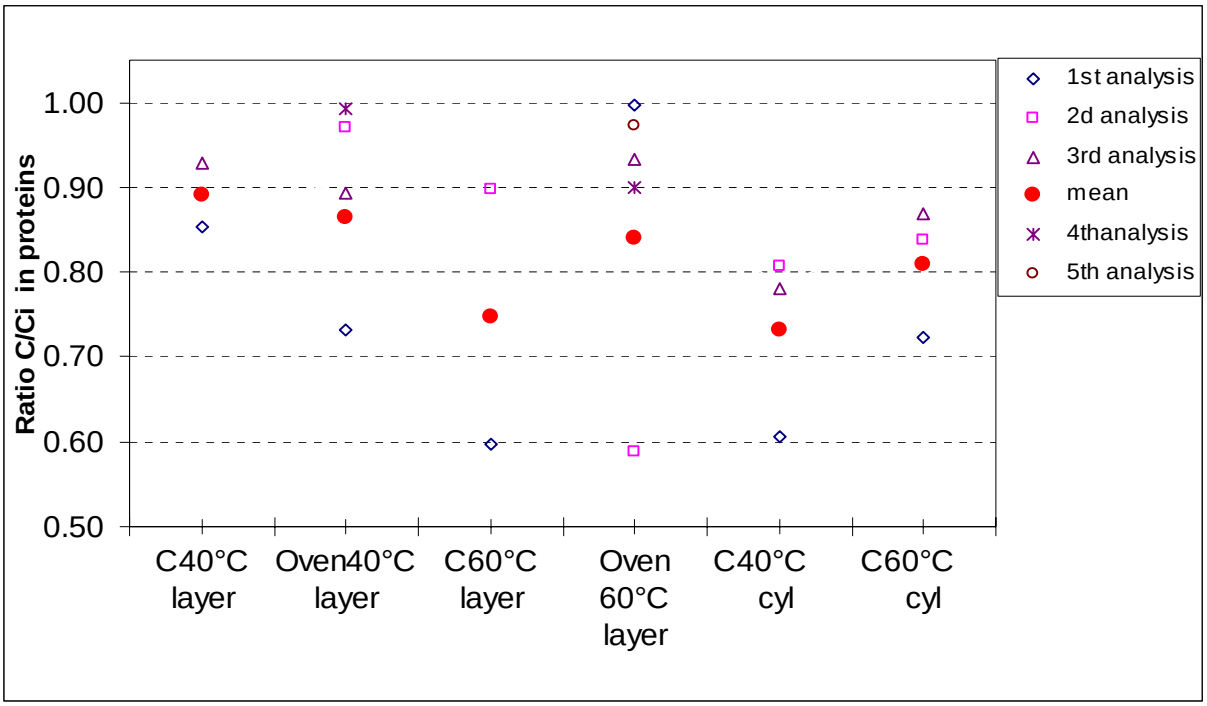


**Figure 14. Influence of convective and oven drying on the loss in protein content from the samples dried in form of layers or/and cylinders.**

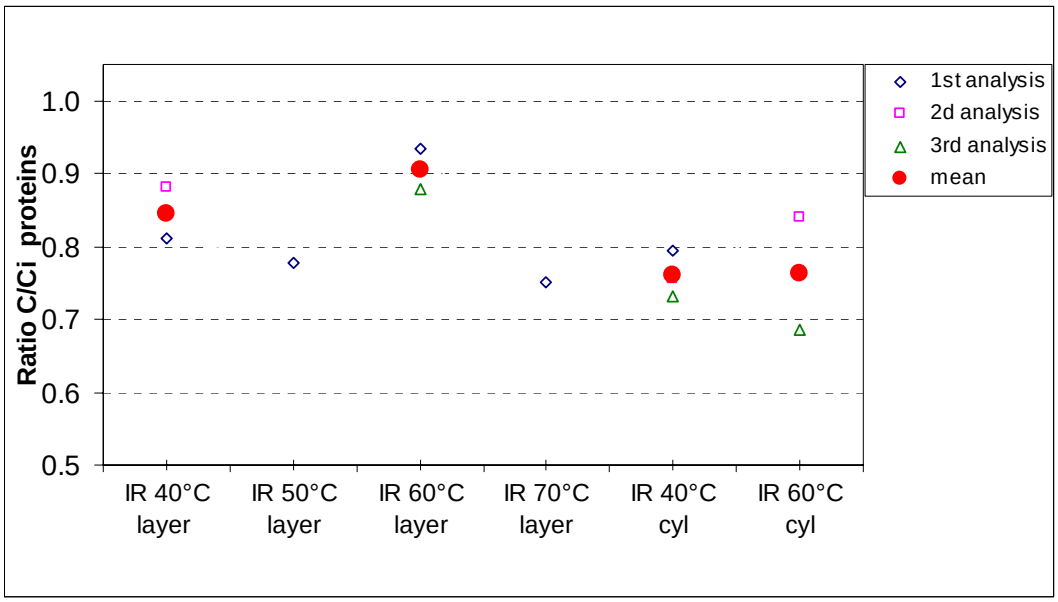


**Figure 15. Influence of the drying temperature of the infrared drying on the form of the samples on the loss in proteins.**

### *3.2.3. Infrared drying*

The results on figure 15 show a loss of 10 to 15% for the *Spirulina* spread out in thin layer and of 25% for measurement in cylinders.

The type of spreading out have a important influence. The reactions on the surface, primarily due to the presence of sugars, intervene in this small loss. When dried by means of the infra-red treatment and in thin layer, the *Spirulina* has on the surface an aspect very shining and "polymerized".

It will be interesting to study the chemical reactions in the *Spirulina* during drying as a function of the time and the temperature to understand the variation of the biochemical (and organoleptical) qualities of the dried *Spirulina*.

### *3.2.4. Spray drying*

Figure 16 shows the results obtained when the spray dry method at 130° and 150°C and respectively 70 and 95°C outlet air temperature is used.

For this type of drying method, only few measurements were carried out. The protein losses are about 15% for the samples dried with 150°C, therefore less than by drying on a support by convection or infra-red. The influence of the temperature has to be studied. The experiments showed that the product has neither the same aspect nor the same colour at 130 and at 150°C.

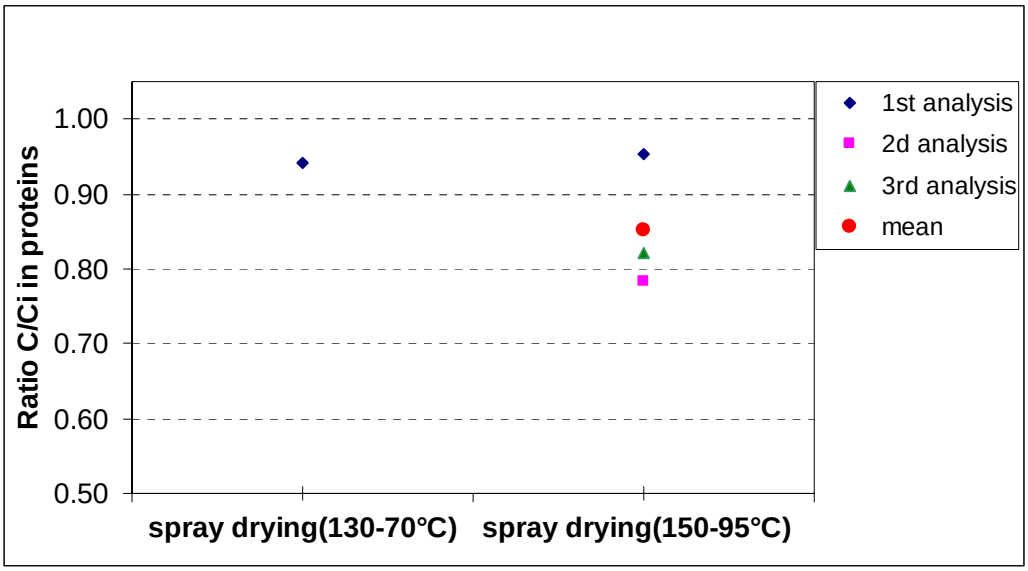


**Figure 17. Influence of 130 and 150°C spray drying on the ratio C/Ci in proteins.**

### *3.2.5. Freeze drying*

All the freeze drying experiments were done at the same operational conditions (see table 1). Figure 17 shows that drying by means of the freeze-drying method causes the weakest protein loss lower than to 10%.

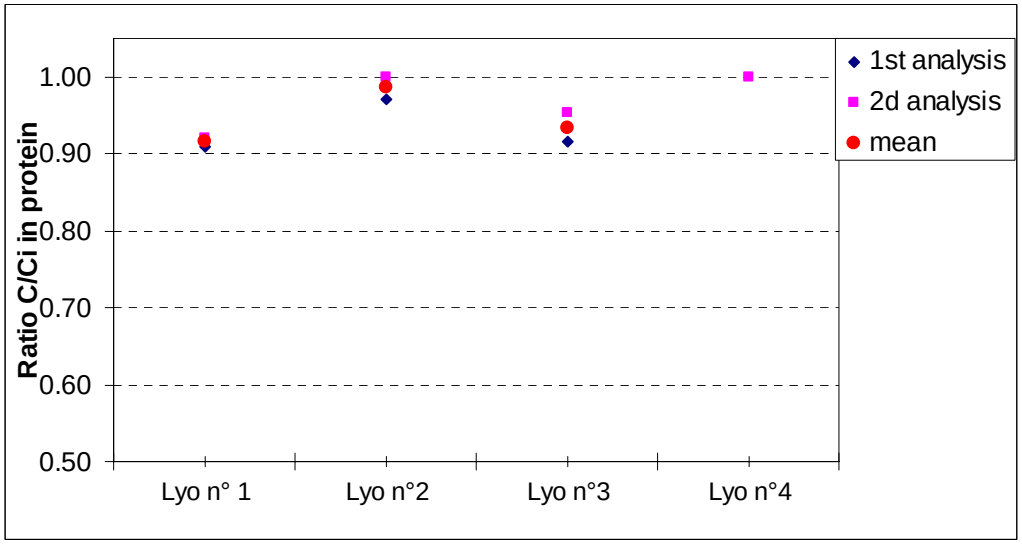


**Figure 17. Influence of freeze drying on the ratio C/Ci in proteins.**

It is interesting to remark that the dispersion of the results of analysis is very weak. The powder product is very homogenous.

### *3.2.6. The protein loss average is function of the process:*

Figure 18 show the proteins loss in function of the drying processes. On this figure, the results obtained with oven drying and those obtained after convective drying are regrouped, due to the low value of the air velocity during convective drying that bring similar drying conditions for the both processes.

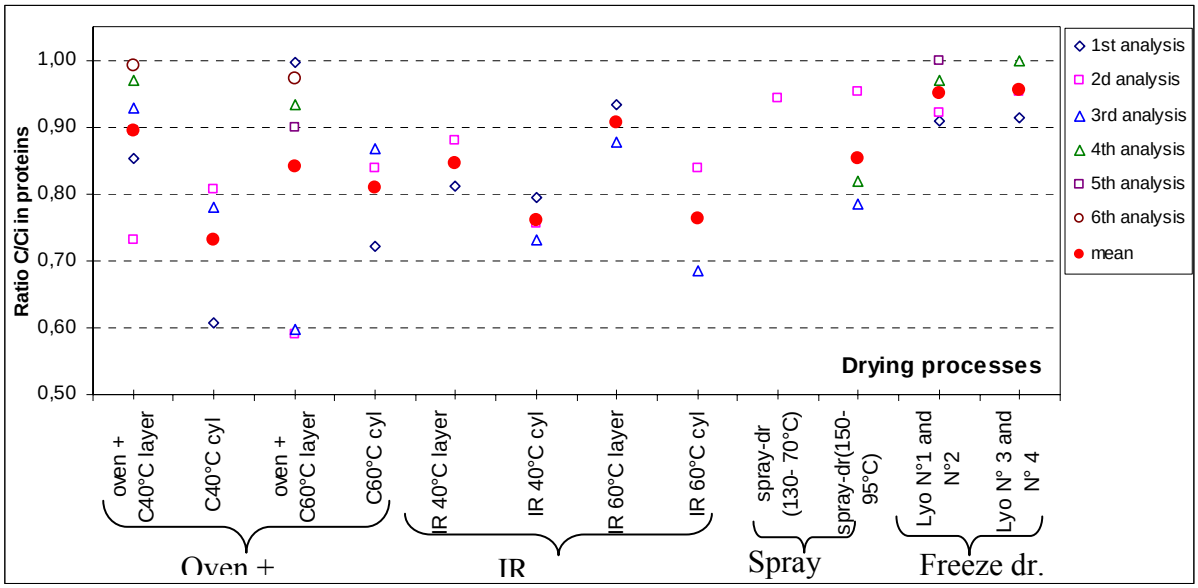


**Figure 19. Influence of different drying processes on the ratio C/Ci in proteins: Oven-drying, convective-drying (C), infrared drying short (IR), spray drying and freeze drying (Lyo).**

The dispersion seems to depend much more on the type of drying. Drying by freeze-drying and by atomization bring a better reproducibility of the results, which is probably due to homogeneity of the dry sample and its form of powder. By convective and infra-red drying, variation in temperature involves phenomena of hardening on surface samples which seems absent for the other processes (spray and freeze drying). Drying thin layer by infra-red, the *Spirulina* surface has an very shining and "polymerized" aspect. Observation show that the exopolysaccharides can be together in some several yellowish small points. Then after drying, the composition in the surface and in the core of the cylinders or along the thin layer can be different. It would be interesting to obtain the protein concentration profile in the thickness of the final product.

Freeze-drying causes the weakest lower protein loss, maximum 10%. The dispersion of the results of analysis is very weak in comparison to the others processes. This can to be due to a

better homogeneity of the product or a better dissolution (characteristic to the freeze-dried products).

By atomization or spray drying (high temperature, short time) where two experiments were carried out, the loss is weak : 10-15%. The experiments showed that the end product does not have the same aspect and color by 130 and 150°C. It will be necessary to carry out other measurements to study the air temperature effect.

Drying at 40°C by hot air (convection or drying oven) corresponds to "soft" conditions and low protein losses of about 10-15%.

Infrared drying on thin layer brings weak loss, 10 to 15% but 25% loss for experiments with cylinders form. It seems that the reactions on the surface, primarily due to the presence of sugars, intervene in this weak loss.

Although the spreading out in cylinders or thin layer involves a great dispersion, the hardening on the surface can be required for special organoleptic qualities and the possibility of retaining the pigments.

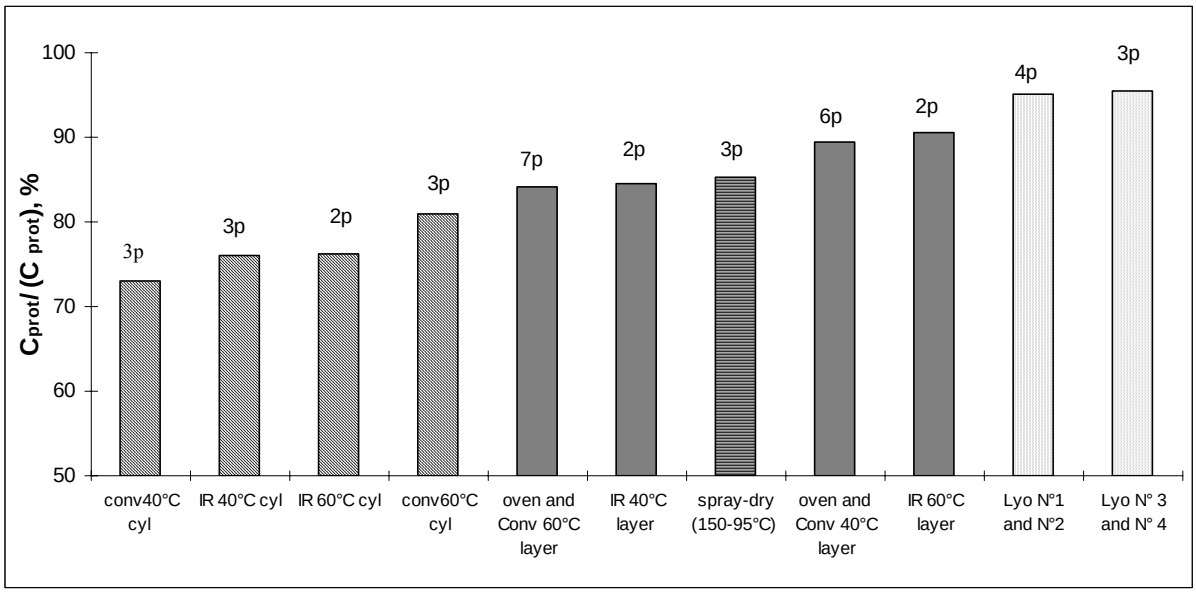


**Figure 19. Influence of different drying processes on the ratio C/Ci in proteins: Oven-drying, convective-drying (C), infrared drying short (IR), spray drying and freeze drying (Lyo). The processes are classified from the higher loss in protein to the lower. The oblique line correspond to the spreading out in cylinders, the full grey for thin layer, the horizontal line for spray drying and the points for freeze drying.**

Figure 19 show the average protein content after drying with the processes that are arranged from the higher loss to the smaller loss of protein (obtain for freeze drying).

It appears clearly that the drying in cylinders cause more proteins losses than the form in thin layers : 20-25%; thin layer 15%.

Although there is no hierarchy of the different drying processes with the criteria of the organoleptic quality , there is lot of text (on the internet sites from small production) which presents the cylinders form as the best form with the best taste to incorporate the *Spirulina* in the food. An advantage of these form is to avoid a great dispersion of the color in the food, due probably to the crust around the cylinders, a hypothesis is proposed that these barrier allows to limit the releasing of the pigments in the food. Further studies could verified this phenomena and measured the composition of the crust.

Then, although no scientific paper has been found to measure these organoleptic qualities, the classification of the processes according to the protein loss criteria is exactly in the opposition of the classification of the process according to the widespread qualities of taste.

Other criteria have to be taken account to choose the best adapted process.

## 3.3. Influence of drying temperature on the total sugars content.

The reproducibility is better for the analysis of one sample than the results obtained with proteins. Figure 20 shows the total sugars loss in function of the drying air temperature, for different samples, taken of at different time of the culture.

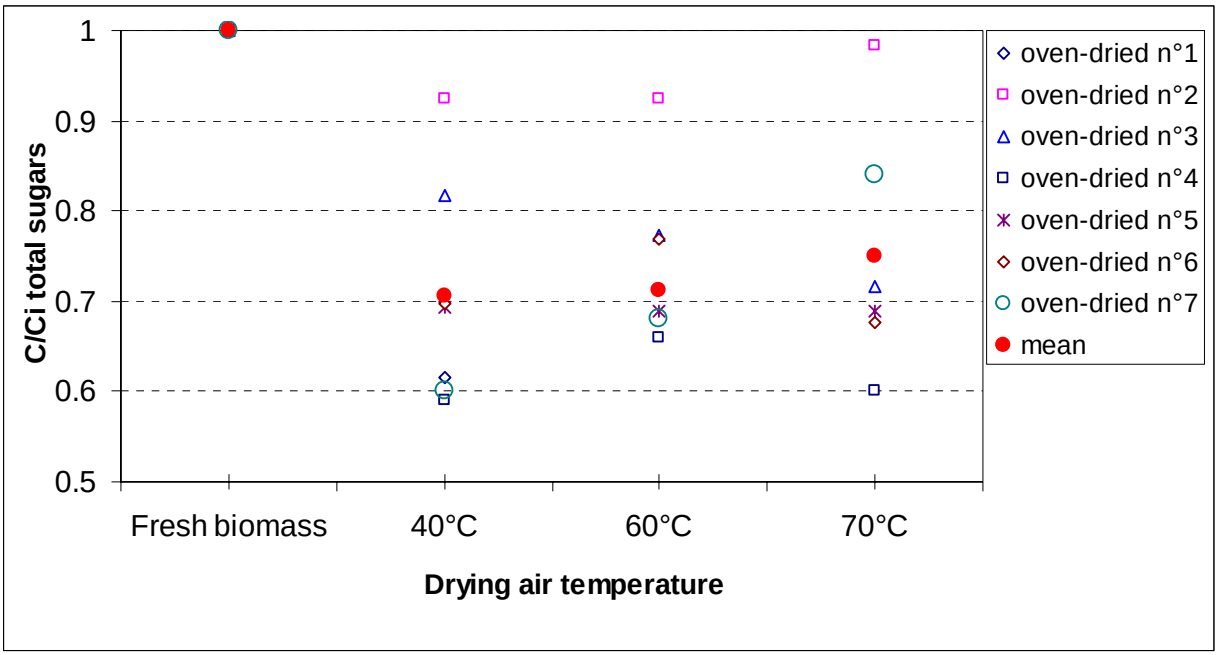


**Figure 20. Ratio "content total sugars after drying/ content initial total sugars" from the samples dried to different temperatures of the air during the oven-drying.**

The results show that the deviation is important and only a tendency can be observed. Except the exp. n°2, most of the analysis show a loss of total sugars from 40°C greater than 30%. Then, the average total sugar loss has a more significant value according to the temperature than that of proteins (average of 10%). A assumption is that the initial sugars concentration can be different from one sample to another. And if the transformation reaction is different at 40°C and at 70°C, the interaction can be different with the chemical reagent. But these assumption has to be verified with several samples taken off at the same time from the culture, homogenised and then dried. The experiment doesn't give a conclusion about the influence of the temperature between 40 and 70°C. According to these results, the limit air temperature to obtained no damage on sugars is perhaps under 40°C by oven drying.

## 3.4. Influence of drying processes on the total sugars content

Figure 21 shows the sugars loss after different drying processes.

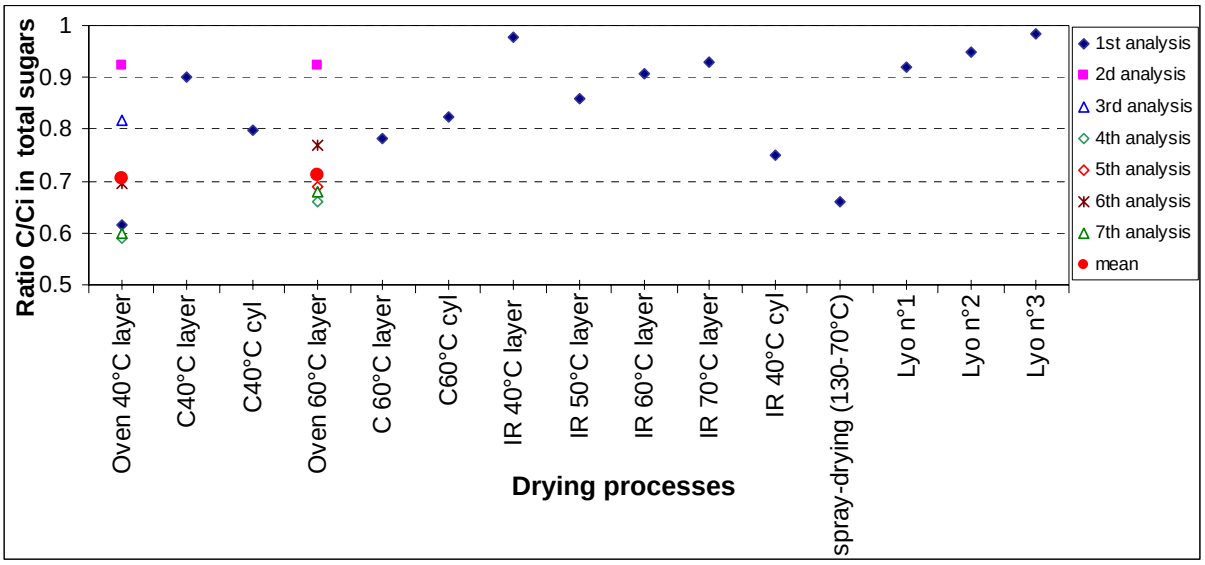


**Figure 21. Influence of different drying processes on the ration C/Ci in total sugars: Oven-drying, convective-drying (C), infrared drying short (IR), spray drying and freeze drying (Lyo).**

The results allow to conclude that the processes can be arranged from the lowest to the highest loss:

The freeze-dried *Spirulina* affects the lowest total sugars diminution, below 10%.

By infrared drying, the 10-15% loss is weak specially for the *Spirulina* spread out in thin layer. This diminution is higher for cylinders form. It seems that the reactions on the surface, primarily due to the presence of sugars, intervene.

Only one results by spray drying is analysis and brings high diminution. But other experiments by atomization will be carried out to confirm this value.

Drying by convection gives a loss of 20 % and oven-drying (not or little circulation of the air) brings to important losses (30%) under any temperature. It seems that the hardening of the product surface, a crust and the form of spreading out has an influence on the variation of the total sugar content. Analysis of the surface product and in the core have to be envisage to verify this heterogeneity.

# 4. Conclusions

The diminution of protein and total sugar content is analyzed after convective drying, oven-drying, infrared drying, spray drying (or atomization) and freeze-drying. The best process for the recovery of proteins and total sugars is freeze-drying which allows for a loss of less than 10% of each analysis. For convective, infrared and oven drying, the decrease is different for the sugars and the proteins. The dispersion of the loss value is in function of the method of the spread-out *Spirulina*. The spreading out in piece like cylinders or layer, allows to show a visible crust on the round for piece of one or two mm thickness. That means that the composition of such dried piece is different between the core and the crust. It is not possible to conclude if small pieces from spray drying presents or not gradient of composition in the thickness.

The results showed that the highest loss of proteins and total sugars is obtained by convective and infrared drying. In a oven drying, according to the air temperature, the total sugar loss (around 30%) is more significant than that of proteins (around 10-20%). Using hot air drying between 40 and 70°C the loss in protein content is proportional to the drying temperature, but the total sugar diminution (loss of 30%) remains constant. The spreading out in cylinders occurs more degradation of analyzable proteins than in thin layer (figure 19). That mean that the type of the drying process is not so important as the arrangement of the *Spirulina* for the protein damage. The arrangement defines the exchange specific area between the air and the product. The analysis of the protein after crushing shows a better repeatability, it shows that the composition is not homogenous in the sample in cylinder or layer form when the initial thickness is greater than 2mm.

Simultaneously, the other parameter for the proteins change and loss after drying is the drying air temperature (fig 10 ) and the couple "air temperature - drying time". The used drying process such convective and radiative drying (IR or other radiation) could be tested with air temperature lower than 40°C (30-37°C is the growth temperature of the *Spirulina*).

Good biochemical properties are obtained using drying processes that provide an end product in powdered form with freeze drying. However, this form of end product doesn't have good organoleptic properties and occurs releasing of colour. Thus, for the choice of a drying process, it will be necessary to take into account the importance of all the intervening factors.

# 5. Perspectives

A new reactor has been build in 2004 and allows higher production of *Spirulina*. The draft has been ended.

One student will be in the laboratory for few months from may 2005. Several possibilities of biochemical analysis are possible, but not previously foreseen to be performed under this Work package:

- The analysis can be repeated and more detailed, by oven drying, in cylinder (for the appreciate taste), by 30 - 40 and 60°C to analysed the effect of the temperature. The analysis can be done after crushing (and one time without crushing). It is possible to use the analysis to study if there is a difference between the surface and inside a piece of dried *Spirulina*. These analysis concerns more the protein than the sugars (is the sugars value of *Spirulina* so important as the protein value? ).

- Others analysis can be carried out on the pigment and on the phycocyanine.

- The amino acids can be analysed. But the literature shows that the evolution of the majority of the amino acids concentration after drying in function of the drying processes is in the same way as the protein evolution after drying.

Nevertheless no draft with ESA or UAB has been detailed at present time.

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