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1 GENERAL INTRODUCTION

In this WP 43.222 different aspects concerning biomass treatment will be studied as described in TN 43.221.

The definition of a procedure for the biomass conservation and preparation for the crew compartment is discussed, and two final possibilities are presented for consideration.

There are as well other factors that should be considered at this point in order to define more precisely the process of preparation of the biomass as food. These are, at least the washing of the cells, in order to eliminate their excess of salts and the treatment of the food to get it free of microbiological activity and with the required degree of water content.

This work is presented in three parts. In the first part, washing procedure is defined. In the second part biomass treatment is considered, and pasteurisation, spray dryer and freeze-dryer procedures are presented. In the third part the effect of these treatments on the biomass quality have been included.

2 PART I-WASHING STEP

2.1 INTRODUCTION

After harvesting step, biomass of *Spirulina platensis*, pass through a washing step, in order to eliminate salts and some undesired compounds such residues from growth medium that could affect main biomass quality.

In TN 43.220 the washing procedure was described, and two alternatives were considered: a membrane filter and a centrifugation system.

Using centrifugation cycles, cell disruption is expected to be higher (see TN 43.21), so, this method has been preliminary discarded and membrane filtration selected. Washing step was carried out with a membrane module after the centrifugation step.

Biomass from the harvesting step was washed with fresh washing solution, with similar pH but lower salinity than biomass suspension. This step was conducted until salt content was reduced at the desired level by conductivity monitoring.

The washing step is schematised in Figure 1:

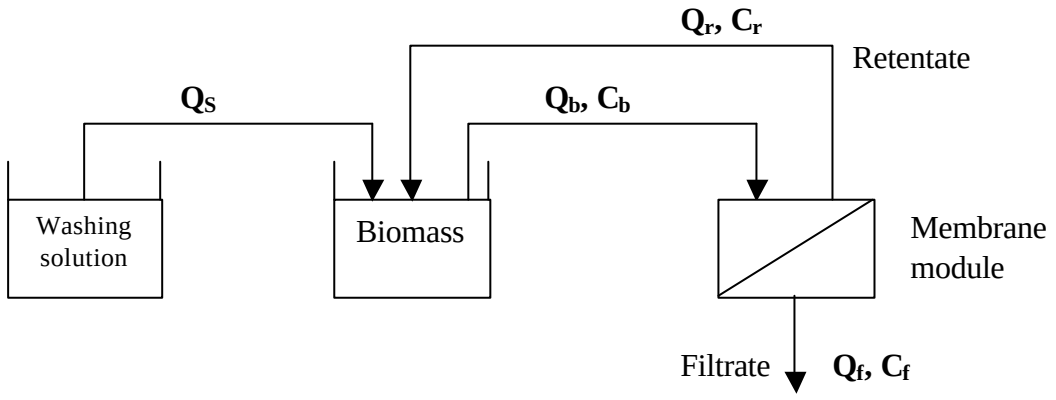

Figure 1. Washing process based on a "Feed and Bleed" operating scheme.

2.2 EQUIPMENT DESCRIPTION AND WORKING CONDITIONS

To perform the washing test, the equipment selected was from *Millipore* and the model used Minitan ^{TM}.
Technical data is as follows:

- Membrane surface net: 60 cm^2/plate
- Number of plates: 4
- Dead volume channel: < 50 ml
- Membrane pore size: 0.65 µm- DVL POPO4-Durapore.

In order to conduct tangential filtration for biomass washing four plates were used. Thus, the total working filtration area obtained was 240 cm^2. In order to reduce membrane clogging by *Spirulina* cells the membrane pore size used was 0,65 µm instead of the standard 0.45 µm-membrane used in previous harvesting experiments. Despite the fact that membrane equipment was discarded for harvesting operations its use to wash biomass appeared as satisfactory enough due to present not only shorter operating time but also total volume to be treated.

Test was conducted for an initial volume of *Spirulina platensis* of 500 ml and a cell concentration of 5.6 g/l (this biomass was obtained from a previous harvesting step). This volume is the corresponding to a complete day-cycle harvesting.
Initial pH and conductivity of biomass suspension were measured and values were 8 for pH and 4.06 mS/cm for conductivity.

As washing solution, 2 litres of water obtained from *Milli-Q plus* filter (*Millipore*) were used. pH and conductivity were 8.7 and 1.51 µS/cm respectively.

As operational variables the following ones were selected as considered suitable to study the general performance and behaviour of the system: inlet pressure (P_e), retentate flow rate (Q_r), filtrate flow rate (Q_f), washing solution flow rate (Q_s), filtrate concentration (C_f) and filtrate conductivity (Cd_f), all being measured. The biomass suspension feed flow rate was fixed and kept approximately constant during the entire test by a peristaltic pump and manually controlling the inlet pressure.

Test was conducted during 3 hours uninterrupted. Every 10 minutes, Qr and Qf were measured and samples from filtrate were collected in order to determine both biomass concentration and conductivity.

Residence time was calculated using the following equation:

$$t = \frac{V}{Q_f} = \frac{500}{10} = 50 minutes$$

V = initial biomass volume (500 ml)
Q_f = approximate filtrate flow rate during the process (10 ml/min). See Table 1.

If a conductivity reduction higher than 95% is desired, total operation time can be calculated by:

$$Cd_f = Cd_i * e^{-t/t}$$

Cd_f = final biomass suspension conductivity
Cd_i = initial biomass suspension conductivity

When considering operation time as $t = 3\tau$, conductivity had to be theoretically reduced to a 5% of its initial value if a well-mixed tank model applies.

2.3 RESULTS AND DISCUSSION

Obtained results for the operational variables selected are presented in Table 1 and Figure 3. Conductivity continuously decreased as expected (exponential form). Salt removal was conducted until a desired value considered high enough according to the previous well-mixed tank assumptions, that is 95%.
During the test, Pe slowly but continuously increased and reached a steady value of about 1.6 bars. During test longed, Qr and Qf decreased only slightly. Thus, future operational problems associated with either permeate flux reduction or severe clogging phenomena can be discarded.
Qr/Qf ratio always was kept higher than 20, normally considered high enough to prevent, or at least reduce, membrane clogging
Qf/A ratio decreased during the test, but not representing neither operational nor efficiency problems.

So, it can be stated that membrane filtration is effective to wash biomass and additionally, it has been shown by microscopically observation that cell disruption is smaller compared to centrifugation system. It can be observed in Figure 2.

In order to quantify or to bear in mind a possible scale-up of the washing operation the volume or amount of biomass that can be treated in this step has to be taken into account. It will depend always on the relation between:

$$\frac{Qf}{A}$$

Where Qf is the biomass feed flow (l/h), and A is the total membrane area in m^2 (240 m^2). The result is expressed in $l/h*m^2$ and this value come up to the process efficiency. In this case is about 25 $l/h*m^2$ as referred in Table 1. However, it is necessary to take into account a new factor, the biomass concentration (X) because, in this way, the biomass load to the system can be calculated. This biomass load over the membrane system is very important since it represents a key parameter in membrane clogging. The higher the biomass load is the higher fouling and clogging of the membrane appears.

So, biomass load can be expressed as follows in units of $Kg/h*m^2$

$$\frac{Qf * X}{A}$$

For scale-up purposes, the value of this quotient will not depend on the used area, but will depend on the membrane retention capability. If alternate cycles of operation/cleaning of the membrane system are considered, the previous relation can be expressed as:

$$\left(\frac{Q_f * X}{A}\right) * t * A_t = \text{total biomass} = V_t * X_t$$

Where t is the operation time, A_t is the total area to be used, V_t the total volume to be processed and X_t the biomass concentration of the suspension to be treated.

Thus, (Q_f*X/A) is always wanted to kept constant and At can be changed to obtain different operating times by only changing the number of membrane plates used whenever biomass X is approximately the same. If higher biomass concentration has to be treated, lower total volume could be processed, because the product V_t*X_t must to be rather constant.

$$\left(\frac{Q_f * X}{A}\right) * \frac{t * A_t}{X_t} = \text{total volume} = V_t$$

In the present experiment, an operation time equivalent to 3τ = 150 minutes for an initial biomass concentration = 6g/l was applied.

If biomass concentration increased until higher values (20g/l obtained in the different centrifugation tests reported in TN 43.21), and not varying total area and volume, Q_f will decrease in a reverse proportion (Qf/4), and t will increase proportionally (t*4= 12h). Thus, taking into account that the operation time can be about 12 hours, when biomass concentration (X) after centrifugation step is maximum, it can be concluded that two complete washing cycles/day could be carry out, if necessary.

Moreover, biomass quality does not decrease before two days in the holding tanks. Thus, the washing step is fast enough to not be the limiting step. In this way, considering that the expected production volume from compartment IV is 20 l/day, the maximal volume that could be treated/batch would be 20 l/day * 2 days = 40 l to the centrifuge. As a result, 1 l of concentrated biomass would be obtained at 20 g/l that could be processed in 24 hours. Another alternative would be to minimise the time between washing cycles, and then, proceed to a washing sequence every 12h for about 6h of operation.

Since the production step is slower than the centrifugation step plus the washing step scale-up and assembly of the three steps may be possible using holding tanks and a batch operation sequence.

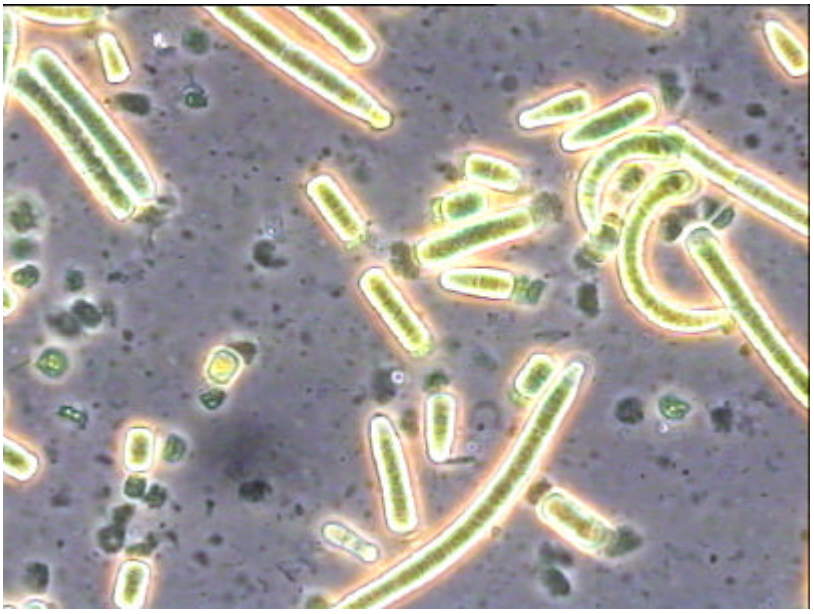

Figure 2. S*pirulina* cells after washing step (*40)

Sample	Time (min)	t/t	Pr (bar)	Qr (ml/min)	Qf (ml/min)	Qr/Qf	Cd (ms/cm)	Abs. (750nm)	Cc (g/l)	Qf/A ($l/h.m^2$)
1	10	0.2	0.2	260	10	26.00	2750	0.0678	0.0406	25.0
2	20	0.4	0.8	260	15	17.33	2490	0.0542	0.0324	37.5
3	30	0.6	1.4	240	12	20.00	2350	0.0680	0.0407	30.0
4	40	0.8	1.4	240	10	24.00	1996	0.0495	0.0296	25.0
5	50	1.0	1.4	240	10	24.00	1725	0.0404	0.0242	25.0
6	60	1.2	1.5	240	10	24.00	1488	0.0583	0.0349	25.0
7	70	1.4	1.5	240	10	24.00	1263	0.0474	0.0283	25.0
8	80	1.6	1.5	240	10	24.00	1056	0.0501	0.0300	25.0
9	90	1.8	1.6	230	9	25.56	944	0.0349	0.0209	22.5
10	100	2.0	1.6	230	9	25.56	837	0.0276	0.0165	22.5
11	110	2.2	1.6	228	9	25.33	757	0.0281	0.0168	22.5
12	120	2.4	1.6	224	8	28.00	690	0.0325	0.0194	20.0
13	130	2.6	1.6	222	9	24.67	631	0.0251	0.0150	22.5
14	140	2.8	1.6	222	9	24.67	573	0.0343	0.0205	22.5
15	150	3.0	1.6	220	10	22.00	491	0.0314	0.0188	25.0
16	160	3.2	1.6	222	10	22.20	437	0.0257	0.0154	25.0
17	170	3.4	1.6	222	10	22.20	405	0.0298	0.0178	25.0
18	180	3.6	1.6	220	11	20.00	370	0.3008	0.0180	27.5

Table 1. Washing test results

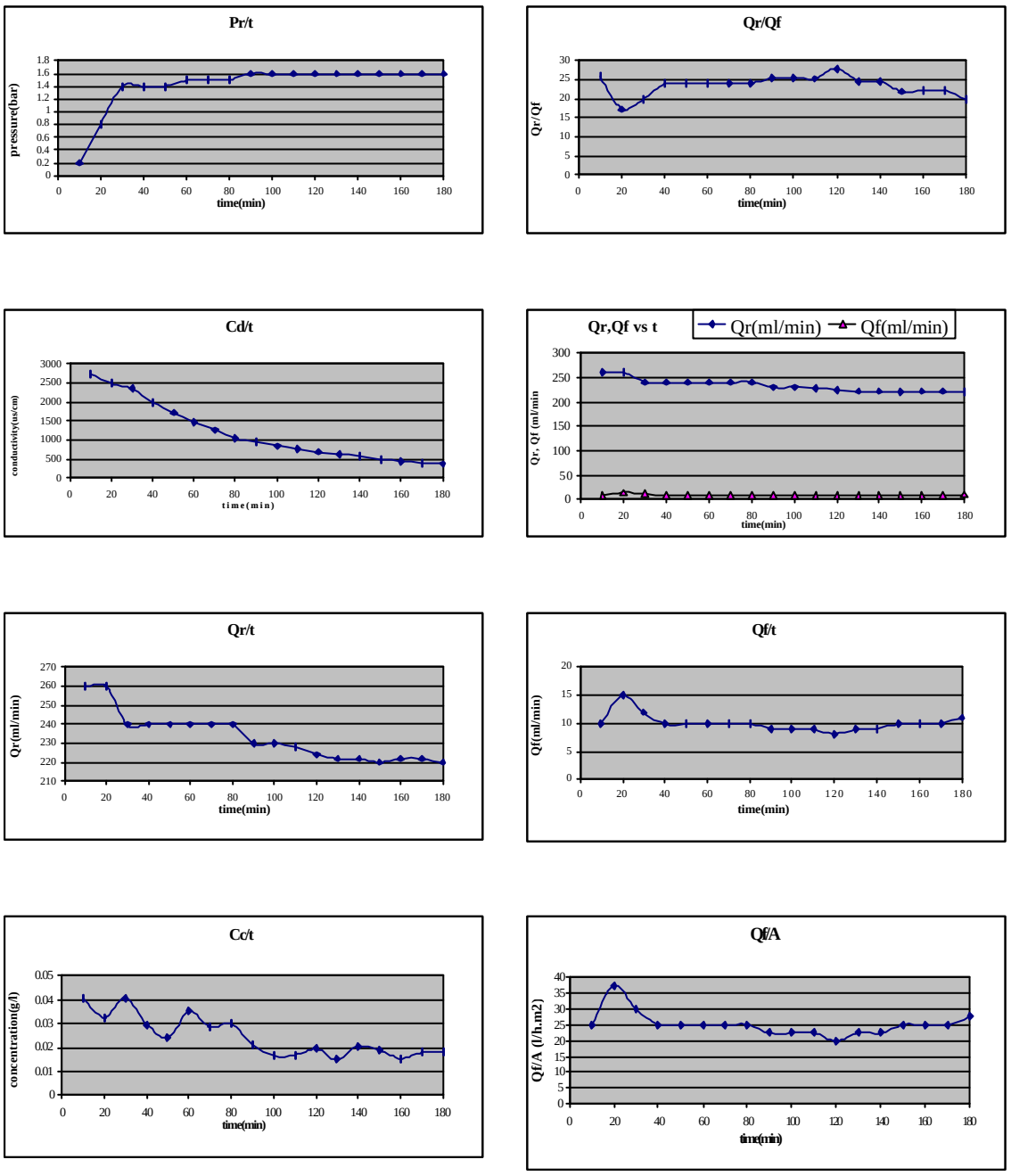

Figure 3. Washing test results plots.

3 PART II- BIOMASS FINAL TREATMENT

4 PASTEURISATION STEP

4.1 INTRODUCTION

After harvesting and washing steps, *Spirulina platensis* biomass suspensions need to be processed through a pasteurisation step in order to be consumable.

In TN 43.220 two pasteurisation processes were defined, depending on selected product presentation: a pasteurisation process to be applied to a liquid product or a dried process (drum dried or spray dried) in which water is eliminated.

In this document pasteurisation process applied to *Spirulina* is defined and results obtained are also presented.

A pasteurisation process assures destruction of pathogenic microorganisms in order to guarantee that the product can be consumed without any risk for human health. At the same time, microbial contamination is reduced and so, product is preserved for a longer period of time (in refrigeration at 4º C), although process does not assure total destruction of microorganisms.

Pasteurisation can be made in two general forms: in a "vessel" type pasteuriser with double wall and a central agitator; and in the HTST method (high temperature, short time).

The "vessel" type pasteuriser works normally at 65 ºC of temperature and a retention time of 30 minutes. This pasteurisation system almost never is used because it needs a lot of time to obtain a good heat exchange and moreover, it is difficult to control the temperature gradient.

This HTST method (high temperature, short time) is better, among other reasons, because it makes easier to calculate the changes that the product experiments while being heated. Quality changes are consequence of the different fluid particles being subjected to different temperature-time conditions. This is the method that was used in the presented experiments.

The HTST work temperature is always < 100ºC and depends on the nature of the product. Pressure is also a factor to be taken into account and it changes depending on the product to be used (more o less viscous). This system frequently uses plate or tube heat exchangers.

The most important goal to achieve in *Spirulina* pasteurisation is to reduce the microbial contamination and assure that the product is healthy. At the same time pasteurisation avoids the losses of some compounds that are found in the *Spirulina* cells while other processes does not.

Work conditions were defined and measures of some parameters were also defined as detailed herein after.

4.2 EQUIPMENT DESCRIPTION AND WORKING CONDITIONS

Equipment used for this HTST pasteurisation can be found in the Food Technology Unit at the Veterinarian Department in the U.A.B. This equipment was designed to work at relatively low volume of product (25-50 litres per hour) and it has three separated sections:

- Recuperation section with a plate heat exchanger.
- Pasteurisation section, where product reaches the maximum temperature and a tubular zone outside the pasteuriser (retention tube) which allows time control. Depending on the tube section the product needs more or less time to flow through.
- Refrigeration section, where feed exchanges heat with the aid of a refrigeration liquid, in this case water and glycol at 4 ºC.

Feed flow-rate was fixed at 50 l/h. Feed was kept into a vessel and pumped to the recuperation section with a PD pump. It was not necessary to work under pressure because feed presented a low viscosity.

Food went out from the pasteuriser at 4 ºC in order to avoid a progressive chilling. Thus, contamination risk decreased.

Recuperation value was approximately of 60%. From initial 25 litres, 15 litres of pasteurised product were recuperated.

In *Spirulina* pasteurisation two work conditions were studied. First, treatment at 76 ºC and 15 seconds of retention time was tested. A second treatment at 76 ºC and 30 seconds of retention time. This temperature seems to be adequate to not produce important quality losses in the pasteurised product. This fact has to be checked by microbiological and quality analyses as presented in the following sections.

Before pasteurisation, biomass was stored in two sterile tanks (one for each treatment) of 25 litres of capacity. Tanks were connected to the outlet of the reactor and biomass harvesting was made in aseptic conditions. Filled tanks were preserved at refrigeration temperature (4 ºC) until their posterior use in the Food Technology Plant.

For every treatment, three samples were collected in sterile glass bottles. One sample was collected at the beginning of treatment, second sample at half time treatment and third sample at the end of treatment.

These samples were used to carry out the microbiological analyses to check whether treatment had been successful and to make quality analyses. If treatment is not enough to reduce microbiological contamination another test with other conditions might be done.

A set of three samples for every treatment was freeze-dried and kept refrigerated in order to make quality analyses, just in case treatment had not been microbiologically satisfactory.

For the treatment with a lowest microbiological contamination content a sample was stored for one week, in order to check the self-life of the product. After this period of time, the sample was freeze-dried.

Pasteurised product (15 litres for each treatment) was collected, in aseptic conditions, in a sterile tank and kept in refrigeration at 4 ºC.

After every treatment pasteuriser was cleaned with water and sodium hydroxide solution until remainders were eliminated.

4.3 RESULTS AND DISCUSSION

In order to check if treatment had been successful, microbiological analyses were made. The ANABIOL Laboratory (Barcelona) was responsible for the realisation of these analyses. Samples were collected and carried under refrigeration (the same day that pasteurisation was made) until its analysis in ANABIOL Laboratory.

Results are presented in Table 2 and a copy is enclosed at the end of this document.

Sample corresponding to 76 ºC and 15 seconds (first treatment) has a higher initial microbial contamination than sample corresponding to 76 ºC and 30 seconds (second treatment). This fact makes difficult the comparison between the two treatments. This contamination difference could be due to the fact that one sample was collected one day after the other, so contamination coming from the reactor could be different and increase during its storage within the thank (thank was sterile).

First treatment reduced the initial contamination one logarithm (total aerobics) and enterobacteria were not eliminated at all. So, this treatment is not severe enough from the microbiological point of view. Probably, results have been negative due to high initial microbiological contamination.

The second treatment handles a sample with lower initial contamination than first treatment. This fact produces some advantages in respect previous sample.

This treatment reduces the initial contamination two logarithms (total aerobics) and eliminates all contamination (enterobacteria and *Streptococcus D*). An aliquot of this sample was also stored in a bottle for a week; then, additional analyses were made to measure self-life. Results on those analyses were positives, because contamination did not increase a lot. So, although this treatment is effective from the microbiological point of view, the higher retention time (30 seconds) produces salt precipitation in the equipment that could damage it.

Pasteurised biomass, in both cases, had a different aspect that fresh initial biomass due to pigments changed of colour (green to brown colour) due to temperature effect.
This effect called "browning of pigments" normally occurs when vegetable food is heated.

Decimal reduction time for the two treatments (time necessary to reduce 90% the initial contamination or reduce one logarithmic cycle) can be calculated and are presented in the following points.

4.3.1 DECIMAL REDUCTION TIME:

General formula:

$$D = \frac{t}{\log X_0 - \log X}$$

Decimal reduction time of the first treatment is:

$$D = \frac{0.25\,\text{min}.}{\log \frac{218000}{15200}} = 0.21\,\text{min}.$$

Decimal reduction time of the second treatment is:

$$D = \frac{0.5\,\text{min}.}{\log \frac{59400}{720}} = 0.26\,\text{min}.$$

Although retention time is twice high folder in the second treatment that in the first one, the decimal reduction time values are not too different. There is only a difference of few seconds between first and second treatment required to reduce the initial microbial contamination. If a comparison between the two treatments is desired, an easy calculation can be made as follows:
A retention time of 30 seconds for the first treatment instead of 15 seconds:

$$D = \frac{0.5\,\text{min}.}{\log \frac{218000}{15200}} = 0.43\,\text{min}.$$

So, for the same retention time, reduction decimal time is higher in the first treatment than the second treatment. Difference is due to the fact that biomass used in the two treatments has a different initial contamination.

New experiments were planned in order to check the effect of cold in salt precipitation and to check whether low retention times (15 s) give satisfactory results when having a low initial contamination.

	76 ºC - 15 seconds			**76 ºC - 30 seconds**			
	Initial	**Half treatment**	**Final**	**Initial**	**Half treatment**	**Final**	**After one week**
Total aerobics (cfu/ml)	218000	23200	15200	59400	1120	720	1060
***Enterobacteria* (cfu/ml)**	59400	740	180	5300	Absence	Absence	Absence
Staphilococcus aureus (cfu/ml)	Absence	Absence	Absence	Absence	Absence	Absence	Absence
***Streptococcus D* (cfu/ml)**	580	Absence	Absence	1840	Absence	Absence	Absence
***Salmonella* presence /25 ml**	Absence	Absence	Absence	Absence	Absence	Absence	Absence
***Pseudomona* (cfu/ml)**	Absence	Absence	Absence	Absence	Absence	Absence	Absence
***Clostridium* sulf. reduc. (cfu/ml)**	Absence	Absence	Absence	Absence	Absence	Absence	Absence
***Escherichia coli* (cfu/ml)**	Absence	Absence	Absence	Absence	Absence	Absence	Absence

Table 2. Microbiological analysis (1) results

4.4 NEW EXPERIMENTS

Previous experiments were repeated in order to check whether the differences found between the two treatments were due to different contamination level present at the initial samples.

Two new experiments were made with the same equipment and working conditions that previously described. In this case, especial attention was paid to achieve homogeneous microbiological characteristics at both samples. That is to say, one sample was collected one day after the other because of production restrictions, but both were mixed before pasteurisation in order to minimise the microbiological differences between them.

4.4.1 RESULTS AND DISCUSSION

ANABIOL Laboratory from Barcelona also carried out microbiological analyses. Results are presented in Table 3 and a copy is enclosed at the end of this document.

From the results it can be observed that the two analysed samples (one for each treatment, first at 76ºC, 15 seconds and second at 76ºC, 30 seconds) have a similar initial microbial contamination. In this case, the initial contamination is much lower than for previous experiments. This may be due to the fact that *Spirulina* bioreactor worked now at the best conditions.

As far as both samples have, in the present test, the same initial contamination, the differences observed in microbiological after pasteurisation must have been due to operational differences in the pasteurisation treatment. That is, different temperature and retention time produced different results.

In both cases, although initial contamination value is very low, initial contamination has been reduced by a factor of 3.5 approximately (total aerobics) and analyses show a total absence of other microorganisms. One sample (76ºC, 15 seconds) was stored in a bottle for a week and then analysed. Results were satisfactory because contamination did not strongly increase. So, pasteurisation is a good method to prepare the biomass to be preserved from contamination, in refrigeration, for a few days

In these new experiments and for the second treatment, corresponding to 76ºC and 30 seconds, salt precipitation from the culture medium did not take place. The reason for this fact is not yet really clear, but it seems that the *Spirulina* reactor worked in such conditions that an excessive addition of base solution or CO_2 for pH control was not necessary (CO_3^{2-} or CO_2 addition).

4.4.1.1 Decimal reduction time

Decimal reduction time for the first treatment (76ºC and 15s.) was:

$$D = \frac{0.25\,\text{min}.}{\log\frac{5960}{1760}} = 0.47\,\text{min}.$$

Decimal reduction time for the second treatment (76ºC and 30s.) was:

$$D = \frac{0.5\,\text{min}}{\log\frac{5900}{1880}} = 1.02\,\text{min}.$$

These values are different, and when retention time is twice fold higher (30 seconds in the second treatment), decimal reduction time is also almost twice fold higher. This means that longer periods than 15 s do not provide better contamination reduction. In other words, the sample do not became less contaminated for longer pasteurisation periods than 15 s.

So, due to the good results obtained related to the microbial contamination reduction, the best treatment would be the one in which biomass quality would suffer less damage. Taken into account the temperature effect in the biomass quality, the weaker treatment seems the best (76ºC and 15 seconds).

Results of biomass quality analyses are discussed in the last part of this TN 43.222.

	76 ºC - 15 seconds				76 ºC - 30 seconds		
	Initial	**Half treatment**	**Final**	**After one week**	**Initial**	**Half treatment**	**Final**
Total aerobics (cfu/ml)	5960	3120	1760	1840	5900	2320	1880
***Enterobacteria* (cfu/ml)**	Absence	Absence	Absence	Absence	Absence	Absence	Absence
***Staphilococcus aureus* (cfu/ml)**	Absence	Absence	Absence	Absence	Absence	Absence	Absence
***Streptococcus D* (cfu/ml)**	Absence	Absence	Absence	Absence	Absence	Absence	Absence
***Salmonella presence* /25 ml**	Absence	Absence	Absence	Absence	Absence	Absence	Absence
***Pseudomonas* (cfu/ml)**	Absence	Absence	Absence	Absence	Absence	Absence	Absence
***Clostridium* sulf. reduc. (cfu/ml)**	Absence	Absence	Absence	Absence	Absence	Absence	Absence
***Escherichia coli* (cfu/ml)**	Absence	Absence	Absence	Absence	Absence	Absence	Absence

Table 3. Microbiological analysis (2) results

4.4.2 GENERAL CONCLUSIONS

According to the results obtained, adequate treatment depends, principally, on the initial microbial contamination of biomass.
When contamination is high, it is recommend a treatment with a higher retention time (30 seconds). When initial contamination is moderate or low both treatments are suitable but a low retention time (15 seconds) is preferred because of the biomass quality preservation.

Results of biomass quality analyses are discussed in the last part of this TN 43.222.

5 SPRAY DRYER STEP

5.1 INTRODUCTION

In the general process of preparation of the biomass as food defined in TN 43.220 two possible final products presentations were defined.
One possibility or proposed presentation is a powdered biomass. In this case, biomass of *Spirulina platensis* is taken after harvesting and washing process and is driven through a spray dryer system. The biomass falls within the spray dryer chamber and the excess of water is eliminated by evaporation. In this process the biomass temperature reaches a value that by a matter of fact biomass becomes almost sterilised.
The product may, then, be vacuum-packed, or under modified atmosphere packed, and can be stored for long time periods (months).

The drying atomisation operation consists in the conversion of one liquid feed in a dried product, in a single and continuous operation. The drying operation includes three stages:

1. Atomisation of feed liquid into a spray by use of an atomiser wheel rotating at high speed or by two a two-fluid nozzle.
2. Contact of the spray with hot air to promote evaporation. The hot air enters the chamber through a ceiling air dispenser.
3. The separation of dried product from the air in a cyclone.

The basic principles involved concern the atomisation of the feed liquid into a spray of very small droplets. These droplets have a very large surface area and evaporation is completed rapidly. The necessary small droplet sizes are produced by rotating the atomiser wheel at high speed or by a two-fluid nozzle, where compressed air creates the atomisation. The resulting evaporation rates are high enough to enable completion of moisture removal from the droplets. During the evaporation stage there is an accompanying cooling effect on the droplets and the drying air, and with limited duration time of the product in the chamber, heat damage of the product is prevented

Atomisation can be made with a rotary centrifuge atomiser or with a pressure nozzle. The system used to dry *Spirulina* is the first one. In this case, hot air is introduced in the drying chamber by an air disperser that surrounds a rotary centrifuge atomiser. *Spirulina* is atomised by means of the centrifuge force generated by a wheel rotating at 8000-10000 rpm.

Product obtained is a powder with a homogenous size that can be stored for a long time, because water contents has been reduced until very low values (3% approximately). Characteristics and quality analyses will be made in order to check the effect of the process and their efficiency.

Detailed definitions of the selected equipment and working conditions are explained herein after.

5.2 EQUIPMENT DESCRIPTION AND WORKING CONDITIONS

Selected equipment was from *NIRO A/S* and the model used was the *Mobile Minor.* Test was made in the UPC (Universitat Politècnica de Catalunya) that gently lent us their equipment.

The *Mobile Minor* spray dryer is designed and constructed to handle small quantities of product. Experience has shown that information obtained on the unit is not totally applicable for planning the scale-up of the drying process, but can give qualitative results to consider their applicability. The spray dryer is simple to operate and control. A drying test run can be quickly set-up, and test powder is available immediately after test drying is started.

Spray Dryer Specifications are:

- Type of dryer: Mobile Minor, Basic Model

- Chamber: diameter 800*620mm/cone.60º, inside sheeting AISI 316, cladding of painted mild steel.

- Exhaust system: Cyclone diameter 140 CHE. Option: Cartridge filter.

- Heating: El-heater, 7.5 kW, and max. inlet temp.350ºC

- Atomising equipment: rotary atomiser, pneumatic driven, with standard or abrasive resistant wheel and/or co-current two-fluid nozzle.

- Powder collection: Single point under the cyclone.

- Pump: Peristaltic pump, infinitely variable control, locally. Power consumption: 0,1 kW.

- Control panel: Included. Cabling to power consumers and instruments included.

- Fan: Exhaust fan. Fan motor: 0.25KW/2900 rpm.

- Material: Parts coming into contact with product are made of stainless steel, AISI 316.

- Noise level: 72 dB (A).

- Drying air rate at 200ºC inlet temp: 80 Kg/h

- Maximal operation temperatures: air inlet 350ºC (662ºF), air outlet 120ºC (248ºF).

- Drying capacity: Between 1-7 Kg water evaporation/h depending on parameter chosen.

- Space requirements L*B*H: 1800*925*2200 mm.

- Weight: 280 Kg.

Principal features of the *Mobile Minor* spray dryer are presented at the end of this document (a copy is enclosed).

The most important parameters used in the *Spirulina* drying operation were:

- Pressure: was fixed at 3,5 bars according to *NIRO* recommendations.

- Speed of rotary atomiser: this parameter is related with inlet pressure. According to pressure/speed diagrams (from *NIRO* manual) speed was set at 20000-30000 rpm.

- Temperature: it is the most important parameter because it has to be low enough to not produce important quality losses in the dry product, but at the same time this value owe to fall within the operating range defined in the equipment specifications. Outlet gas temperature is related with feed flow rate. When feed flow rate (pump speed) increases outlet gas temperature decreases. Inlet air temperature was fixed at 165-200 ºC and the pumped flow was adjusted to 10 rpm. In that way, outlet gas temperature was

90ºC so the *Spirulina* was dried but not over-dried (see capacity diagram from *NIRO* manual at the end to this document).

Before *Spirulina* drying, the Spray Dryer was cleaned with distilled water and sodium hydroxide (3%) in order to eliminate residues and contamination from previous operations. Spray Dryer was run in this way for few hours until a total disinfection.

After that, *Spirulina* was pumped into Spray Dryer with a peristaltic pump *(Watson Marlow* model) being the feed flow rate about 1 l/h. This flow rate is quite low, but it could not be risen (in the spray dryer used) because of drying capacity limitations. However, this flow rate is expected to be high enough to treat the concentrated biomass obtained from the centrifugation system. The harvesting system is planned to be operated to produce 0.5 l/day-1.0 l/day of concentrate.

Spray Dryer was run in a continuous mode for 5 hours and steady state for optimal conditions were obtained after 30 minutes of operation, that is:

- Outlet air temperature: 90ºC.
- Inlet air temperature: 200ºC.
- Pump speed: 10 rpm.
- Feed flow rate: 1 l/h.
- Pressure: 3,5 bars.
- Rotary atomiser speed: 20000-30000 rpm.

5 litres of *Spirulina* were treated and the powder product was collected in a glass jar under the cyclone.

Every hour this glass jar was replaced in order to collect the powder, so five samples were obtained once the process was ended. Every sample was stored and kept refrigerated in order to make the quality analysis.

Finally spray dryer was cleaned with distilled water and sodium hydroxide solution (3%) until remainders were eliminated.

5.3 RESULTS AND DISCUSSION

Result of drying process is a powder with homogenous characteristics, very thin granulometry (droplet size) and very low humidity. This size is related with the dryer chamber diameter. The *Mobile Minor* Spray Dryer is laboratory equipment of small dimensions and this fact, determine the product size.

The product obtained was analysed to measure microbial contamination and the effect of this treatment in the biomass quality parameters (proteins, carbohydrates, lipids, and nucleic acids contents).

Microbiological analyses are presented in Table 4 and a copy is enclosed at the end of this document (analyses were obtained from ANABIOL Laboratory).

The results show that spray dryer process allow obtained a product microbiologically correct, due to temperature effect during the operation time. This effect is comparable to a low sterilisation.

Results of biomass quality analyses are presented and discussed in the next chapter.

	RESULTS
Total aerobics (cfu/ml)	42400
Enterobacteria (cfu/ml)	Absence
Staphilococcus aureus (cfu/ml)	Absence
Streptococcus D (cfu/ml)	Absence
Salmonella (presence in 25 g)	Absence
Pseudomonas (cfu/ml)	Absence
Clostridium sul. red. (cfu/ml)	Absence
Escherichia coli (cfu/ml)	Absence

Table 4. Microbiological analysis results.

6 FREEZE-DRYER STEP

6.1 INTRODUCTION

Even though freeze dryer process it is not considered and studied as a possibility for biomass treatment (pasteurisation and spray dryer methods have been tested) this method has been used for drying and preservation of the *Spirulina* samples to be analysed. So, it is considered that this procedure needs a short explanation since it has been used as a standard reference method.

It is demonstrated that freeze dryer is a good alternative to dry *Spirulina* biomass because gives a high quality, and non-degraded product but it is probably the most expensive method (Ripley, 1996). So, most commercial alga growers use spray-drying process.

In general, freeze-dryer process is a special kind of dehydration by sublimation on ice from the food in water vapour. The difference between this method and the others dehydration methods is that the modifications in the foods are minimum. Also, freeze dryer only need a soft heating, so the nutritional and sensorial characteristics of the final product are similar to the fresh product. Nevertheless, dehydration speed is slow and the equipment and operation cost (low temperatures and vacuum) are high.

Freeze dryer requires a previous step, which is freezing the product. The objective is to transform the aqueous solutions in the foods in a two-phase mixture: one phase formed

by the ice glass and other by the concentrate solute solution. Type and freeze speed will affect final product structure because its porosity will depend on the size and location of the glass ice that are formed. (Ordoñez et al, 1998).

The four basic requirements in the freeze drying process are:

- Freezing: Rapid cooling of the product to a point below its eutectic.
- Vacuum: Removal of air and other non-condensable vapours from the chamber to facilitate vapour migration
- Heating: Carefully controlled heat input to the frozen product to speed the drying process
- Condensing: Trapping of water vapour molecules in the form of ice on the condenser surface.

6.2 EQUIPMENT DESCRIPTION

Selected equipment for freeze dryer the *Spirulina* samples was from *VIRTIS SENTRY* and the model used was *Benchtop 5L*.

The following is a brief description of the mechanical systems in the *Virtis* freeze dryers responsible for the four basic requirements of freeze-drying: (from *Virtis* user manual)

- **Freezing**: The main reason to pre-freeze a product is to lock its particles into a solid matrix so that water and others solvents can be removed without causing physical or chemical changes in the compound. Freezing the product to below -40ºC is usually sufficient. Virtis and 5 Litre use manifold heads for drying. Pre-freezing for these units are done separately by using an external source, or a shell bath.

- **Vacuum**: The purpose of the vacuum system is to continually evacuate all non-condensable gases from the drying chamber. This reduces the resistance encountered by water vapour molecules migrating from the product to the condenser. It also encourages the vapour pressure differential necessary for sublimation and helps to prevent oxidation by removing air.

- **Heating**: When flasks are dried on a manifold, the ambient (or room temperature) air surrounding the flasks wills normal supply enough heat. Heat is applied to the frozen product to maintain vapour migration from the product to the condenser, supplying the necessary energy to drive the vapours, insuring continued sublimation.
- Freeze-drying occurs in two phases: primary and secondary drying. Primary drying is characterised by sublimation from totally frozen product. Secondary drying is continued desorption of trapped moisture until desired consistency.

- **Condensing**: When water vapour molecules leave the product ice they migrate toward the low-pressure areas in the vacuum system. The *Virtis* condenser is helical in shape and made of 304 stainless steel.

7 PART III - BIOMASS QUALITY

7.1 INTRODUCTION

In life support systems, algae have the potential to provide air rehabilitation and food. Some problems are associated with the use of algae as a food, principally the adequacy and acceptability of algal-derived food.

Various criteria of quality for the utilisation of biomass as human food should be developed, including some aspects of nutritional quality and toxicological criteria. In particular: approximated chemical composition, biogenic toxic substances (microtoxins, other toxins), non-biogenic toxins (wastes from cultivation and processing), biochemical nutritional studies, safety evaluations (feeding trials in animals) and acceptability. (Becker 1981, Borowitza 1988, Ciferri 1983, Ripley and Fox 1996, Ortega 1991).

Data on the chemical composition of biomass give key basic information on the nutritive potential of biomass. In addition, this proportion can be modified by specific cultivation such as composition of the culture medium, and light intensity.

Evidence of the nutritional quality is only one of the basic requirements for successful utilisation of biomass in food preparations. Equally important is to guaranty the toxicological safety of the material. Some toxicological investigations have been successfully performed jointly with a satisfactory nutritional quality achievement by several authors (Ortega 1991, Saiz et al. 1993, Tranquille and Emeis 1997).

Critical biomass components have to be within specifications as defined by the corresponding analyses in order to have enough precision and consistency of the data obtained.

Some compounds may change depending on treatment and preservation processes, therefore in some cases additional analysis will required to verify final product quality, in addition to microbiological analysis.

In the present part of the work, the effect of pasteurisation and spray dryer processes in the biomass quality were studied. For that, biochemical composition analyses of biomass were made and results compared with a standard in order to check if treatment can affect the nutritional value of *Spirulina* preparation. Microbiological aspect has not been treated in this part due the fact that results are included in the previous parts to this TN 43.222.

7.2 MATERIALS AND METHODS

7.2.1 SAMPLE PREPARATION

After pasteurisation and spray dryer processes, samples were taken and freeze dried. Freeze dried samples were the following:

Pasteurisation: -One sample of biomass treated at 76ºC and for 15 seconds **(P1)**
-One sample of biomass treated at 76ºC and for 30 seconds **(P2)**
-One sample of P1 stored under refrigeration (4ºC) for a week **(P1w)**
-One sample of P2 stored under refrigeration for a week **(P2w)**

Spray dryer: -One sample corresponding at the end of the drying process (after 4 hours from the beginning t=0) **(SD)**

Standard: -Corresponding to fresh biomass taken directly from the outlet of the bioreactor (not treated and not stored) **(S).**
-Corresponding to biomass stored for two days and freeze-dried **(SPI).**

Quantity taken for each sample was one litre of biomass suspension.
Biomass was centrifuged in order to separate culture medium from *Spirulina* cells (*Beckman centrifuge, model J2-21M/E*) for 15 minutes at 4ºC and 10.000rpm.

Pellet was collected and washed repeatedly in order to eliminate salts from growth medium that could affect biomass quality. Procedure was the following:

Firstly, cells were washed with a phosphate buffer (pH=8.5-9 and concentration=50mM) and centrifuged for 15 minutes at 4ºC and 10.000 rpm.

After that, cells were washed with NaCl (0.85%) and centrifuged for 15 minutes at 4ºC and 10.000 rpm.

Finally, cells were washed with a NaCl + water and centrifuged for 15 minutes at 4ºC and 10.000rpm.

Obtained biomass, without salts, was freeze-dried (*Virtis Sentry, model Benchtop 5L*). (See freeze-dried process description) and stored in refrigeration under sterile conditions until their use.

7.2.2 METHODOLOGY

Every analysis was made for all samples (7 at all) at the same time; for example, protein determination was made for all samples simultaneously and, in the same way with carbohydrates, lipids, etc.

Main biomass compounds that could be more affected by the treatment were analysed, that is to say, which their percentage in the total composition decreases after the biomass treatment (pasteurisation and spray dryer in this case). The different analyses and the methodology used were the following:

- Total protein (*BCA protein assay reagent*, Pierce)
- Total carbohydrates (*Phenol method*, Herbert 1971)
- DNA (perchloric acid extract)
- RNA (KOH extract)
- Chlorophyll and phycocyanin (extraction with acetone and K-Phosphate buffer solution)
- Total lipid content and fatty acids composition (capillary gas chromatography)
- Aminoacids composition (liquid chromatography HPLC)

Most of these methods were described in detail in TN 43.221. In fatty acids and aminoacids composition determination, methodology has been changed in respect to TN 43.221 and a copy of the used protocol is included in the annex to this TN 43.222.

7.3 RESULTS AND DISCUSSION

Obtained results corresponding to freeze dryer, (**S, SPI** or standard sample, not treated by heating), pasteurisation (**P1, P2, P1w** and **P2w** samples) and spray dryer (**SD**) samples are presented in Table 5 and in Table 6.

	S	SD	SPI	P1	P2	P1W	P2W
Proteins (%)	61± 9	58± 9	55± 3	54± 6	53± 9	47± 5	52± 2
Aminoacids (E/NE)	0.65± 0.05	0.76± 0.10	0.72± 0.10	0.74± 0.10	0.73± 0.02	0.74± 0.05	0.74± 0.08
Carbohydrates(%)	13.4± 0.6	11± 2	11± 2	10.7± 0.6	11± 3	10± 2	10± 2
Lipids	*	*	*	*	*	*	*
DNA (%)	3.2± 0.8	2.3± 0.6	2.3± 0.6	2.4± 0.6	2.5± 0.6	2.6± 0.7	1.8± 0.4
RNA (%)	3.6± 0.4	1.6± 0.2	4.0± 0.5	1.0± 0.3	1.1± 0.2	1.0± 0.2	1.8± 0.4
Chlorophylls (%)	0.30±0.02	0.31± 0.02	0.19± 0.02	0.39± 0.02	0.28± 0.02	0.35± 0.02	0.47± 0.02
Phycocyanins (%)	7± 2	2.5± 0.9	3.3± 0.9	0.7± 0.2	0.32± 0 .01	0.38± 0.01	0.8± 0.3

Table 5. Biomass quality analyses. Percentage of macromolecules (% dw). Freeze-dried and not treated by heating (S, SPI), spray dried (SD), pasteurised (P1, P2, P1w, P2w). (E/NE)= ratio between essentials and not essentials aminoacids. * Lipid analyses will be explained as fatty acids.

It must be pointed out that, results corresponding to both pasteurisation and spray dryer processes have been compared in front of standard results. The standard used was a sample taken in the outlet of the bioreactor and immediately freeze-dried, (neither stored nor treated). Biomass used for pasteurisation was initially stored in holding tanks for two days. Thus, samples were not considered as "fresh biomass" and in order to know its quality before pasteurisation they were freeze-dried **(SPI).**

According to the results obtained, it is obvious that any treatment that implies a temperature increment will modify the chemical composition of *Spirulina* in higher proportion than other treatment that uses a temperature not so high.

Freeze dryer seems to be the best alternative if a product with high nutritional quality is desired, as reported in the literature and corroborated by the analyses conducted. However, the main problem is the cost of this method when scale production is large. Anyway, treatments selected and used in these experiments were not very damaging, because work temperatures can be considered as "soft", and so, quality losses were not important.

Obtained values for each parameter lets more or less into a correct range, so quality is adequate in the main part of the samples analysed.

AA	S	SD	SPI	P1	P2	P1W	P2W
Leu	5.7±0.6	4.2±1.4	3.5±1.6	3.0±1.0	3.4±0.2	3.4±0.2	4.0±0.1
Lys	2.8±0.4	2.2±0.7	2.9±1.3	2.7±0.9	3.0±0.1	3.0±0.2	3.4±0.1
Ile	3.2±0.5	3.0±1.3	5.5±2.6	5.0±1.7	5.6±0.2	5.8±0.4	6.5±0.1
Met	1.40±0.05	1.6±1.0	1.0±0.5	1.0±0.4	1.00±0.04	1.00±0.06	1.30±0.04
Phe	2.5±0.5	2.2±0.9	2.6±1.2	2.4±0.8	2.7±0.1	3.0±0.2	3.00±0.03
Thr	2.8±0.4	3.1±1.7	2.5±1.2	2.9±1.0	2.8±0.1	2.8±0.2	3.7±0.1
Trp	0.15±0.01	0.03±0.01	0.09±0.01	0.03±0.01	0.04±0.20	0.04±0.01	0.04±0.05
Val	3.1±0.6	3.1±1.4	3.5±1.7	3.2±1.1	3.6±0.1	3.7±0.2	4.2±0.1
Total essentials	21±1	19±3	22±3	20±3	22.0±0.5	22.5±0.7	26.2±0.3
Ala	3.8±1.4	3.4±0.8	4.8±2.2	4.4±1.5	5.0±0.2	5.0±0.4	5.6±0.2
Arg	4.5±0.2	3.3±0.8	4.6±2.0	4.2±1.5	4.7±0.2	4.8±0.3	5.4±0.1
Asp	5.2±0.9	5.3±3.3	5.1±2.2	4.6±1.6	5.0±0.2	5.1±0.4	6.0±0.2
Cys	0.25±0.02	---------	---------	0.13±0.05	0.15±0.01	0.6±0.6	0.1±0.1
Glu	6.6±1.2	3.1±1.0	4.3±1.9	3.8±1.3	4.3±0.2	4.3±0.3	5.0±0.2
Gly	2.6±0.3	2.7±1.0	3.5±1.6	3.0±1.0	3.4±0.1	3.5±0.3	3.9±0.1
Hys	1.1±0.2	1.2±0.8	1.1±0.5	1.1±0.4	1.25±0.05	1.3±0.1	1.40±0.05
Pro	3.1±0.6	2.5±2.5	2.8±1.3	2.5±0.9	2.8±0.1	2.9±0.2	3.2±0.1
Ser	3.3±0.3	2.0±1.2	1.6±0.7	1.6±0.5	1.70±0.06	1.6±0.1	2.00±0.06
Tyr	2.5±0.5	1.80±0.05	0.1±0.1	2.0±0.7	1.95±0.07	1.0±1.4	2.8±0.1
Total not essentials	32.0±2.0	25.0±5.0	30.1±4.7	27.0±3.0	30.0±0.5	30.0±2.0	35.0±0.5
Quotient (E/NE)	0.65±0.05	0.76±0.10	0.72±0.10	0.74±0.10	0.73±0.02	0.74±0.05	0.74±0.08
Amn (%)	0.8±0.1	0.5±0.3	0.7±0.3	0.5±0.2	0.60±0.02	0.60±0.05	0.60±0.02

Table 6. Aminoacid composition for the different samples analysed (g/100g d.w.). Amn= ammonium percentage.

If results corresponding to every treatment are compared considering the selected quality parameters, the following general conclusions can be stated:

- **Elemental composition:** The elemental composition for the **(S)** sample was the following:

$$CH_{1.74\pm0.01}O_{0.619\pm0.005}N_{0.196\pm0.001}S_{0.005\pm0.001}$$

Comparing this result with the reported in literature one can say that elemental composition has a high variability depending on the operation conditions, mainly when carbon, nitrogen and illumination sources are limited. Thus, Cornet *et al.* (1992) measured the elemental composition of *Spirulina platensis* giving as C-molar formulae for the active biomass $CH_{1.650}O_{0.531}N_{0.170}S_{0.007}P_{0.006}$ which includes extracellular polysaccharide. From several batch and continuous cultures, in different photobioreactors and with different incident illuminations, Cornet *et al*. (1994) also reported the following chemical formulae $CH_{1.58}O_{0.46}N_{0.17}S_{0.007}$. More recently Cornet *et al*. (1998) proposed a structured stoichiometric equation for *Spirulina platensis* considering as chemical formulae for the cyanobacteria the following one: $CH_{1.583}O_{0.514}N_{0.154}S_{0.007}P_{0.005}$.
On the other hand, Filali *et al*.(1993) found the elemental crude formula of exopolysaccharide corresponding to $CH_{1.65}O_{0.95}S_{0.015}$. Thus, it can be expected that different chemical composition for bacteria appears depending on the EPS percentage and sample treatment methods used.

- **Proteins**: Normally represents a value ranging 55-70% of total dry weight. In the standard sample, this percentage is about 62% and is lower in samples thermally treated. In sample **SPI** percentage is lower than sample **SD,** due to the fact that this biomass was stored for two days in a holding tank. When pasteurisation takes place in a shorter time (**P1**) protein percentage is a little higher than for a longer time (**P2**). When pasteurised samples are stored for a week percentage decreases still more (**P1w, P2w**). In spray dryer sample (**SD**), percentage is higher because in this process, little degradation is produced because the operating temperature is lower than 200ºC and operating time less than 2 seconds.

- **Aminoacids**: aminoacid content was determined by HPLC. Results are presented in Table 6. A total of 18 aminoacids were quantified. Among them, Cys and Trp were not detected with a very good resolution with the method used. Nevertheless, results are included in the table.
Values obtained for each aminoacid are in good agreement with the bibliography (Ortega, 1991; NTS ingredients, 1999), and the most important in percentage are Glu, Arg, Ala, Leu and Asp. Although, in general, the sulphur aminoacids as Met and Lys are present in low quantities in the blue-green algae, in the analyses carried out Met was found in low quantity but Lys was found in remarkable quantity.
Spirulina has an important quantity of essential aminoacids, so the *Spirulina* protein has a high nutritional value. In the table of results, essential and non-essential aminoacids have been calculated separately in order to compare samples and calculate the essential/non-essential ratio (E/NE).
In general, one can observe that the E/NE ratio is relatively high, being in the major part of the samples about 0.65-0.76. These values are in good agreement with the bibliography (NTS ingredients, 1999).

If the aminoacid content is compared taking into account the sort of thermal treatment performed, one can conclude than the atomiser sample (**SD**) has a low variation compared with the standard (**S**), and its composition in essential and non-essential aminoacids is a little lower than the standard. Pasteurised samples, (**P1, P2**) have an aminoacid content lower than their standard (**SPI**). Moreover, it is difficult to compare both samples in terms of aminoacid content, and therefore to compare both pasteurisation treatments (**P1, P2**). Perhaps, the main difference is found when the **P2w** sample is compared with the **P2** sample, where the aminoacid percentage in the first is increased respect to the second, probably because of microbial contamination.

Finally, if the different methods are compared considering the total quantified protein for each sample (Table 5), one can conclude that the discussion made in the case of proteins is also suitable for the aminoacid content of the samples.

- **Carbohydrates**: represent a 10-17% of total dry weight. Results discussion is rather the same than for proteins. Heat can affect carbohydrate percentage, as results show. But, in this case it is not clear whether pasteurisation process affects in the same way carbohydrate percentage and proteins.

- **Nucleic acids:** From the literature *Spirulina* has a nucleic acid content about 4-6%. It is known that elevated amounts of uric acid in humans may produce toxicity problems when intake is higher than permissive values. Obtained results have demonstrated that variability is higher for RNA than for DNA, and as reported in the literature, nucleic acids are very sensitive to changes in temperature.

- **Fatty acids:** lipids represent a 6-10% of total dry weight. *Spirulina* contains large amounts of polyunsaturated fatty acids, which makes it an interesting property as food supply. Unsaturated and saturated fatty acids relation (uns/sat) has an important effect in the quality definition of a food product. In the nutritional aspect (low cholesterol) *Spirulina* has an important amount of unsaturated fatty acids. Due to this fact *Spirulina* is a good nutritional supplement.

  A total of five fatty acids were quantified. Of these, four were saturated, that is: palmitoleic, a fatty acid similar to γ-linoleic (triple essential acid), linoleic (essential) and oleic acid. The remaining fatty acid was unsaturated, palmitic acid. (Table 7).

F.A	S	SD	SPI	P1	P2	P1W	P2W
palmitoleic	1.90 ± 0.01	2.10 ± 0.03	0.80 ± 0.14	0.9 ± 0.06	1.50 ± 0.12	1.30 ± 0.06	1.60 ± 0.02
triple ess*	11.8 ± 0.1	13.50 ± 0.04	5.80 ± 0.06	8.5 ± 0.02	9.90 ± 0.04	10.00 ± 0.11	10.60 ± 0.01
linoleic*	9.40 ± 0.01	10.10 ± 0.06	5.40 ± 0.04	5.8 ± 0.02	8.40 ± 0.04	7.50 ± 0.12	9.00 ± 0.05
oleic	2.10 ± 0.05	3.60 ± 0.14	1.60 ± 0.01	1.5 ± 0.06	2.80 ± 0.08	2.30 ± 0.11	2.80 ± 0.11
total uns	**25.2 ± 0.2**	**29.3 ± 1.8**	**13.6 ± 0.7**	**16.7 ± 0.5**	**22.6 ± 1.1**	**21.1 ± 2.3**	**24.0 ± 0.9**
palmitic	18.00 ± 0.01	21.4 ± 0.1	11.00 ± 0.06	10.90 ± 0.02	17.40 ± 0.06	15.20 ± 0.08	17.08 ± 0.09
total sat.	**18.0 ± 0.2**	**21.4 ± 2.4**	**11.0 ± 0.6**	**10.9 ± 0.2**	**17.4 ± 1.0**	**15.2 ± 1.3**	**17.8 ± 1.6**
Quotient (uns/sat)	**1.40 ±0.02**	**1.40 ± 0.17**	**1.24 ± 0.10**	**1.53 ± 0.05**	**1.30 ±0.10**	**1.39 ± 0.19**	**1.35 ± 0.13**

Table 7. Fatty acids composition (mg/g). (* Essential fatty acids).

From the table of results it can be observed that in all samples the fatty acids are in decreasing proportion according to the following order: palmitic acid is the most abundant followed by the acid with three doubles bonds (triple essential), linoleic acid, oleic acid and finally palmitoleic acid.
Samples **S** (standard) and **SD** (spray dried) have a larger amount of fatty acids than the remaining samples specially the last one (**SD**).
SPI and **P1** samples have lower fatty acids amount specially the **SPI** sample.

In order to properly compare the different fatty acids, amount of each fatty acid was divided by the total fatty acids amount (unsaturated + saturated). The obtained results are shown in Table 8.

F.A	S (%)	SD (%)	SPI (%)	P1 (%)	P2 (%)	P1W (%)	P2W (%)
palmitoleic	4.4 ± 0.01	4.1 ± 0.08	3.2 ± 0.8	3.2 ± 0.3	3.7 ± 0.2	3.6 ± 0.2	3.80 ± 0.03
triple ess.*	27.30 ± 0.01	26.60 ± 0.07	23.5 ± 0.1	30.80 ± 0.05	24.70 ± 0.06	27.5 ± 0.1	25.30 ± 0.02
linoleic*	21.70 ± 0.01	19.90 ± 0.08	21.9 ± 0.1	21.00 ± 0.05	21.00 ± 0.06	20.6 ± 0.2	21.50 ± 0.03
oleic	4.90 ± 0.05	7.1 ± 0.1	6.5 ± 0.1	5.4 ± 0.2	7.0 ± 0.1	6.3 ± 0.2	6.7 ± 0.1
palmitic	58.30 ± 0.01	42.20 ± 0.07	55.3 ± 0.1	60.50 ± 0.04	56.50 ± 0.06	58.1 ± 0.1	57.40 ± 0.03

Table 8. Percentage of each fatty acid referred to the total unsaturated + saturated. (* essential fatty acids).

From this table it can be observed that palmitoleic acid has a percentage in all samples between 3.5%-4.5%, the acid with three doubles bonds between 23%-30% approximately, etc. Differences previously observed are reduced and it can be stated that the standard sample has a higher amount of fatty acids than other samples. However, it is difficult to compare the samples corresponding to spray dryer and pasteurisation treatments in quality terms. Probably, changes in the fatty acid composition when biomass has been thermally treated were not important. But, on the other hand, when sample experiment oxidation reactions, this fact produces significant composition differences among them (sample **SPI** was stored for two days in a buffer tank and consequently, this sample and both **P1** and **P2** samples have high differences from the standard sample **S**)

- **Chlorophyll and phycocyanins**: their percentage presents a high variability depending on the culture conditions. According to the literature, chlorophyll values are about 1%. Obtained results showed in general good agreement with it. Anyway, this parameter is difficult to quantify because in the lipid extraction some pigments can also be extracted and so, affecting the final results. Values obtained in the phycocyanin measurements are higher than for chlorophylls because the extraction method is not the same and the dried methods can produce a decrease about one 50% of his percentage (Sarada *et al*, 1998) .It is important to remark that their degradation and colour change "browning of pigments" is more important when the temperature is higher. This effect is higher in the pasteurisation process than in spray drying.

7.4 CONCLUSIONS

One can conclude that there are some parameters to take into account when biomass is treated to use it as food. These are, time that biomass has been stored prior being treated, temperature used during the treatment and time which this temperature is applied.

All these factors will characterise the gross chemical composition of algal food product (ash, lipids, carbohydrates, protein, etc), this is to say, chemical characteristics of the products could be explained considering events related to production procedures.

From the tree methods used, freeze-drying, pasteurisation and spray drying, it can be concluded that the first is the most recommend because product is practically not degraded and biomass quality is higher (sample **S**).

Spray drying (sample **SD**) is a commercial method widely used and product quality obtained is also high (less than freeze-drying). Another advantage is that product has a uniform appearance and it is easy to package.

Pasteurisation is the less expensive method but however, not only damages the product but also food consumption needs to be in a short period of time after the treatment (samples **P1, P2** and **P1w, P2w**).

When biomass is stored in a holding tank, quality decreases as has been proved with the analyses performed (**SPI** sample has important differences in respect to both atomised and pasteurised samples).

All methods are microbiologically effective (see Table 3 and Table 4) but pasteurised product is more easily contaminated due to its higher water content.

From an organoleptical point of view, spray dryer product could be more accepted than a liquid product obtained from pasteurisation.

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ANNEXES