International Journal of Food Science and Nutrition Engineering 2015, 5(3): 101-114
DOI: 10.5923/j.food.20150503.01

An Evaluation Study of the Cultivation of Spirulina in the Area of Sambirano, Madagascar

Zafilaza Armand[1], Andriantsimahavandy Abel[1], Ramamonjisoa Daniel Joseph[1], Andrianainarivelo Mahandrimanana[2,*]

[1]University of Antananarivo Madagascar, Faculty of Sciences, Department of Fundamental and Applied Biochemistry
[2]University of Antananarivo Madagascar, Faculty of Sciences, Department of Inorganic Chemistry and Physics Chemistry

Abstract The area of Sambirano is very favourable to the cultivation of Spirulina. The climates, salinity, the pH, the structure of the ground allow the cultivation of Spirulina. The results show that the rates of the components are not differing in the South from Madagascar. Therefore, to ensure and stabilize the components, it is necessary to set up the method HACCP and the strict method of conservation.

Keywords Spirulina, Physico, Chemical Analysis, Microbiological Study

1. Introduction

Sambirano is in the northewest, District of Ambanja in the region DI.A.N.A. The climate is rainy. The pH, the salinity and the turbidity are favorable to the cultivation. Spirulina is easy to cultivate but requires adequate parameters to develop. A potential problem of *Spirulina* production in open system is the hazard of water contamination with pathogenic organisms. Handling of the product during processing, harvesting and drying, can also result in microbial contamination. The final microbial load of the product will therefore depend on how carefully the culture and product are handled at the various stages of production [7]. Only good manufacturing practices and direct analysis of microbial flora as well as concentration in each lot of product can guarantee the safety of the product. The final product should meet microbiological standards set by the various national and international standards [7, 14].

The production of high quality *Spirulina* therefore requires the use of high-grade nutrients and routine analysis of heavy metals in the culture medium and the product. This is particularly important in situations where food-grade *Spirulina* is produced from open-ponds or natural lakes [7, 23, 41].

Our study is based in the two more or less different cultivations. One is cultivated in the natural environment (Mn) and the other in the improved natural environment (MnA). That's why the main aim of this work is physicochemical analysis of the two samples and follow-up the microbiological analysis. This study is divided into three parties. Firstly, bibliographical study. Second is devoted to the methods of analysis followed by results of the parameters physicochemical and microbiological before and after the conservation. Third, the discussion and finally the conclusion.

* Corresponding author:
m.andria@moov.mg (Andrianainarivelo Mahandrimanana)
Published online at http://journal.sapub.org/food

2. Material and Methods

2.1. Description of the Study Area

Sambirano is an area in the Northwest of Madagascar, in the region of Diana. The name refers to the Sambirano Valley as well as the Sambirano River which runs from the foothills of the Tsaratanana Massif into the Ampasindava Bay where it joins with the Ramena River, south of Ambanja. Due to the proximity of the Tsaratanana mountain range and trade winds, a particular micro-climate occurs in the Sambirano region. During the rainy season, the river floods and deposits extremely fertile alluvia along its river banks, providing ideal conditions for many types of crops, especially cacao.

The rainfall is approximately, 2,000 mm per year in the Sambirano area in the northwest. The temperatures at higher elevations are mainly moderate, between 15° and 25°C. There is a cool, dry season between July and September and a warmer wet season during the rest of the year.

2.2. Morphology and Taxonomy

a) Morphology

Spirulina is symbiotic, multicellular and filamentous blue-green microalgae with symbiotic bacteria that fix nitrogen from air. Spirulina can be rod- or disk-shaped. Their main photosynthetic pigment is phycocyanin, which is blue

in colour. Spirulina are photosynthetic and therefore autotrophic. Spirulina reproduce by binary fission. The helical shape of the filaments (or trichomes) is characteristic of the genus and is maintained only in a liquid environment or culture medium. The presence of gas-filled vacuoles in the cells, together with the helical shape of the filaments, result in floating mats. The trichomes have a length of 50 to 500 µm and a width of 3 to 4 µm. Under light microscopy, the blue-green non-heterocystous filaments, composed of vegetative cells that undergo binary fission in a single plane, show easily visible transverse cross-walls.

Filaments are solitary and free floating and display gliding motility. The trichomes, enveloped by a thin sheath, show more or less slightly pronounced constrictions at cross-walls and have apices either slightly or not at all attenuated [11, 16].

Spirulina is characterized by its regularly coiled trichomes. Under some conditions of temperature and pressure, its helical filaments can convert to abnormal morphologies, such as irregularly curved and even linear shapes, that are considered as a permanent degeneration that could not be reversed. However, the linear filaments of *Spirulina platensis* could spontaneously revert to the helical form with the same morphology as the original filaments. The ultra-structural, physiological, and biochemical characteristics of linear filaments are different from those of the original filaments, whereas they are the same for the reverted and the original filaments [40].

Spirulina is naturally found in tropical regions inhabiting alkaline lakes (pH 11) with high concentration of NaCl and bicarbonates [31].

b) Taxonomy

According to the classification in Bergey's Manual of Determinative Bacteriology, Spirulina belongs to the oxygenic photosynthetic bacteria that cover the groups Cyanobacteria and Prochlorales, which are, by phylogeny, related to the sequence of the rRNA (ribosomal ribonucleic acid) sub-unit 16s. As a function of the sequence data of this sub-unit and the rRNA sub-unit 5s, these prokaryotes are classified within the Eubacteria group.

3. Qualitative Analysis

The spirulina is cultivated in the North of Madagascar beside mangrove swamp called "Fasira".

The chemical analysis of Spirulina is done in two more or less different samples. One sample coming in the improved natural environment and the other comes in the natural environment. The two samples are analyzed by spectrophotometer u.v. or atomic absorption spectrophotometer [6, 8, 13, 15, 32].

4. Risk Evaluation Microbial Contamination

a) Studied Samples

- powder of Spirulina coming from the natural environment (Mn)
- powder of Spirulina coming from the improved natural environment (MnA).

b) Types of the studied microbes

– aerobic flora mésophile total with 30°C
– Coliforms totals with 30°C
– Escherchia colib-glucuronidase (+) with 37°C
– Staphilococca coagulases (+) with 37°C
– bacteria sulfitoreducer with 37°C
– yeasts and moulds with 25°C
– Salmonella [3-5]

c) Mother solution

One takes 10g of Spirulina and to add 90g plugged peptoned water. The unit is to crush during 60s with the STOMACHER. After the solution mother is sudden a serie of decimal dilutions b) Way of calculating – in-depth sowing [2-5].

$$N = \frac{\sum a}{V - n.d}$$

$$N = \frac{\sum a}{V(n_1 + 0.1\, n2)d}$$

ou

$$N = \frac{\sum a}{V - n.d}$$

N: Number of colonies
The sum of Ufc in two dilutions
V: Volume of inoculum ensemenced
n1: number of limp of the 1st dilution
D: factor of dilution corresponds to the 1st dilution
n2: numbers of limp of the second dilution
N: number of limps

– sowing on the surfaces [2-5] for the Staphilococcus coagulase (+)

$$N = \frac{\sum a}{V\,.1.1.F}$$

$$a = \frac{b^{c}}{A^{c}} + c^{c} + \frac{b^{nc}}{A}\,.\,C^{nc}$$

Ac: many mended characteristic colonies
Anc: many mended noncharacteristic colonies
bc: many colonies of positive characteristics of supposed Staphilococca
bnc: colonies number noncharacteristic of supposed Staphilococca positive
DC: many colonies characteristic of Staphilococca positive supposed for limp
Sum of the columns of positive Staphilococca with coagulase identified in two limp.
F: bypass ratio with the 1st dilution
V: volume spread out over each limps

d) Method of enumeration of total aerobic flora mesophile

One takes 1 ml of the two marine solutions and his decimal dilutions coming from the two differents cultivation (Mn and MnA). They are put in limp of Petri. After one makes run 15 ml of PCA in limps and the unit is mixed while turning at least 6 times. After the mixture, let solidify the culture. Incubation is done with 30°C during 72 hours [3-5].

e) Method of enumeration of yeasts and moulds

One takes 0.1 ml of solutions marinates (SM) and his decimal dilutions of the two different cultures (Mn and MnA). The unit is inoculated on the surface of limp of Petri. Container of cultivation OGA. They are incubated at room temperature (25°C) during 5 days. The reading of the colonies is between 15 and 300 [4, 5].

f) Method of enumeration of the anaerobic bacteria sulfitoreducer

One takes 5 ml of the marine solution (SM) of the two cultures (Mn and MnA) and to put in a tube of 20 mm. After, make a dilution of 10^{-1} and take 5 ml of the dilution of the two cultivation and put in a tube of 20 mm. Run in a tube approximately 20 ml of TSC and the unit is carefully mixed while turning the wrist at least 8 times. After this mixture, let solidify them. Incubation is done with 37°C during 24 to 48 hours in the study.

g) Method of enumeration of Escherichia coli-glucuronidase

One takes 10 ml of SM of the two different cultures and to put in six limp of Petri in the proportion of 2 limp X 1.6 ml and 4 limp X 1.7 ml.

Secondly, one takes 10 ml of decimal dilution of the two different cultivation (Mn and Mn A) and to put in six limp of Petri in the proposal of 2 limps X 1.6 ml and 4 limp X 1.7 ml.

In two cultures, make run approximately 15 ml of cultivation TBX in limp of Petri. The unit is mixed while turning at least 6 times the wrist. Let it solidify, the incubation is made with 44°C during 24 hours in the drying oven [5].

h) Method of enumeration of coliforms total

One takes 2 X 1 ml of SM coming from the two different cultivation (Mn and Mn A). They are senescences in two limps of Petri.

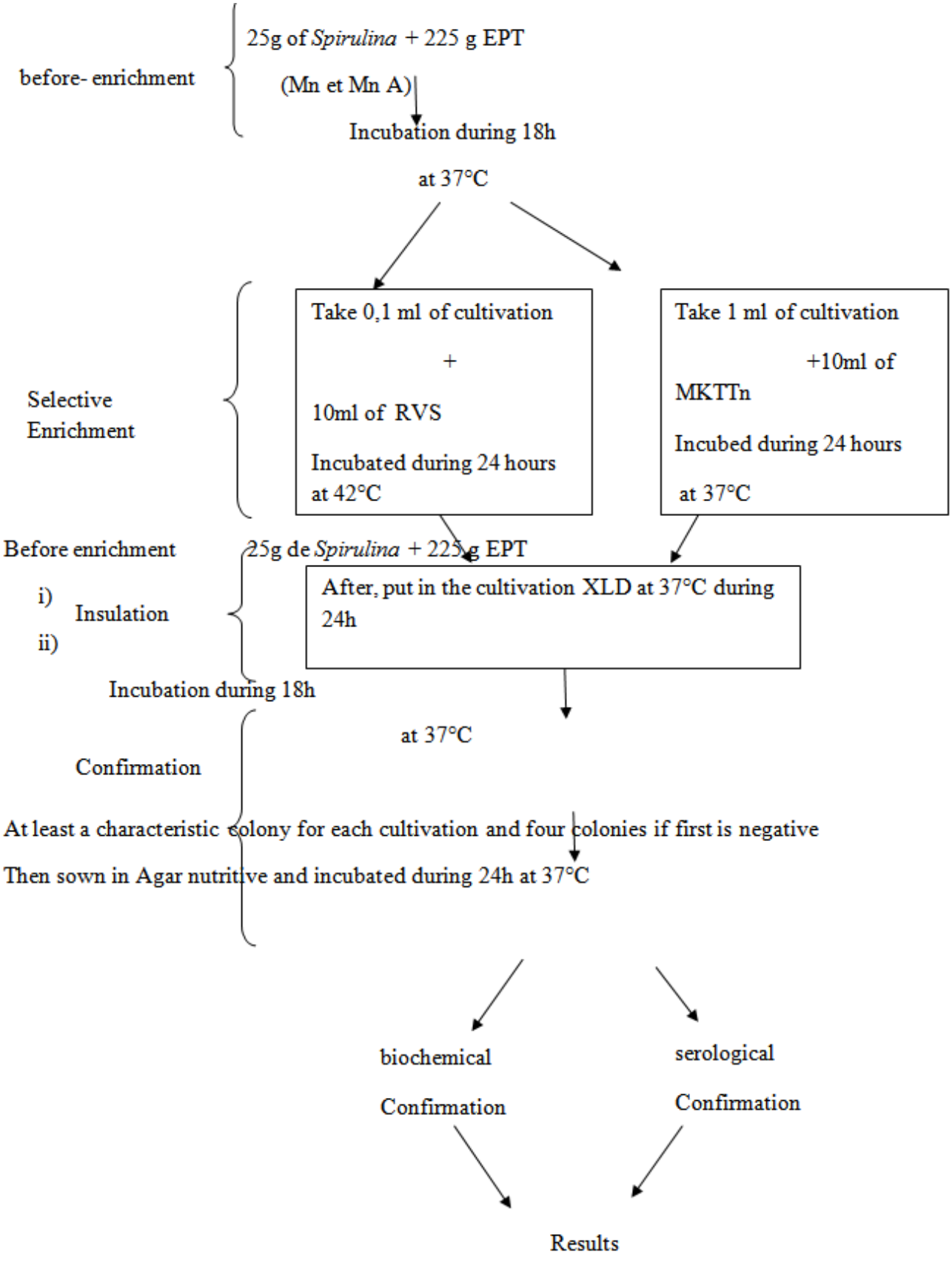
Then, make a dilution 10-1 and take 2 X 1 ml of two dilutions in different cultures to sow in 2 limp of Petri.

In two cultures, make run approximately 15 ml of culture VRBL. The unit is mixed while turning at least 6 times the wrist. Let the unit solidify itself and after addition a second layer of in-top culture. Let it solidify, the incubation is done with 30°C during 24 hours in the drying oven [3].

i) Method of enumeration of positive Staphilococca coagulases

One takes 0.1 ml of SM coming from the two different cultivation (Mn and MnA). And make run in limps of Petri containing of cultivation BP (Baird-Parker agar). Incubation is done with 37°C during 48 hours [2-5].

j) Method of enumeration of Salmonella

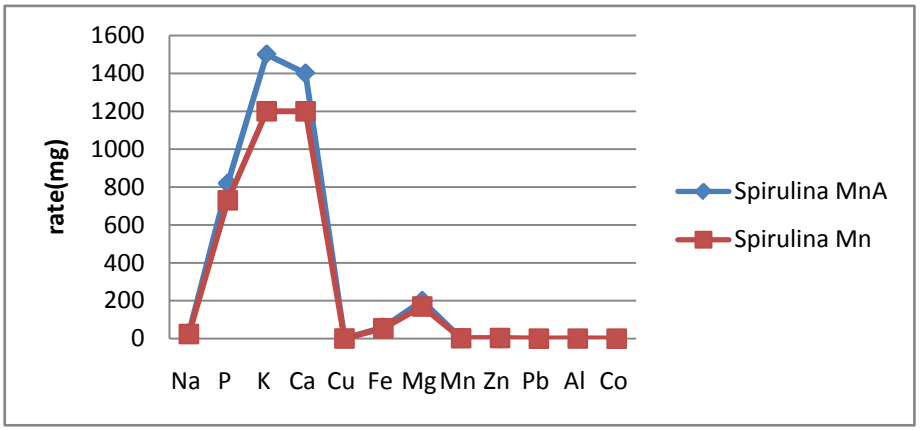


5. Results of Analyses

Qualitative analysis before the conservation

a. Rates of minerals in 100g of Spirulina

Elements(mg)	*Spirulina M_nA*	*Spirulina M_n*
Na	30	25
P	820	730
K	1500	1200
Ca	1400	1200
Cu	0,5	0,9
Fe	60	55
Mg	200	170
Mn	2	2,5
Zn	2,5.	3,5
Pb	0,2	0,4
Al	0,3	0,7
Co	0,1	0,2



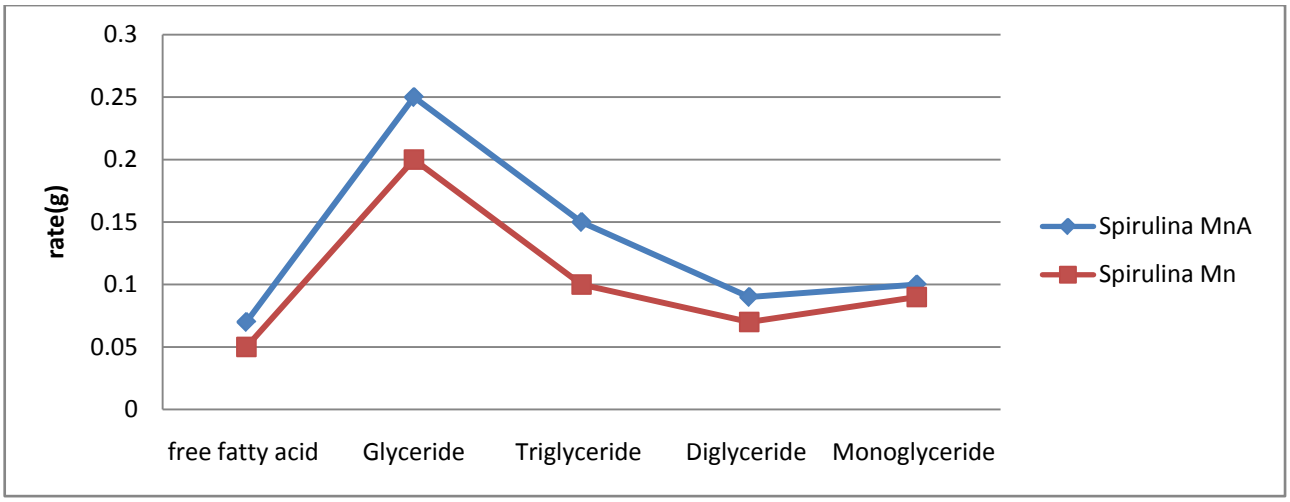
The results show that the rates of the minerals K, That, Na, Mg, P in cultivated Spirulina in the improved natural environment are higher compared to the natural environment. But, for heavy minerals like Pb, Al, Cu, Co, Zn, the rates are higher in Spirulina than the natural environment compared to Spirulina cultivated in the natural environment improved except for Iron (Fe).

According to the analysis the differences are due to the levels of water change is the cleanliness of culture. The water sea renewal after each harvest improves of rate salinity and the pH which influences on the level of parameters of composition of minerals in Spirulina. As in the improved natural culture the rate of salinity and pH is stable. Thus, the formation of minerals inside Spirulinase unroll normal.

If rate salinity and pH vary, the mineral formation varies too. However, the variation of formation is due to the pollution of the culture which causes the increase in heavy metals in Spirulina cultivated in the natural environment. Thus the stability of the mineral rates present in Spirulina depends of the physicochemical parameter of the culture.

b. Rates of Lipids in 100g of Spirulina

Lipids (g)	*Spirulina MnA*	*Spirulina Mn*
Free fat acids	0,07	0,05
Glycerids	0,25	0,20
Triglycerids Diglycerids	0,15	0,10
Monoglycerids	0,09	0,07
	0,10	0,09

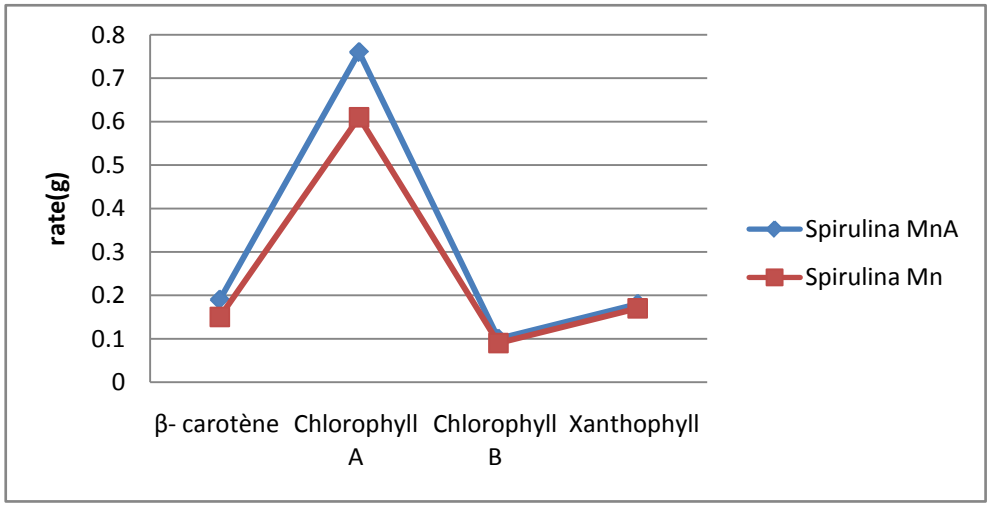


The content of fat in Spirulina cultivated in the improved natural environment is higher by Spirulina report cultivated in the natural environment. The difference between of the two cultures is due to the level of sea water, i.e. physicochemical parameter of the affected culture influences it the formation of the components of the cells.

c. Rates of pigments in 100g of Spirulina

The table shows some trace or the presence of pigments in Spirulina.

Elements (g)	*Spirulina MnA*	*Spirulina Mn*
β- carotenoid	0,19	0,15
Chlorophylle A	0,76	0,61
Chlorophylle B	0,10	0,09
Xanthophylle	0,18	0,17



The β- carotenoid is always the majority pigment in Spirulina. One notices also the presence of high rate of chlorophyl A compared to Chlorophyl B.

The rate of 4 identified elements is varied from culture to the other.

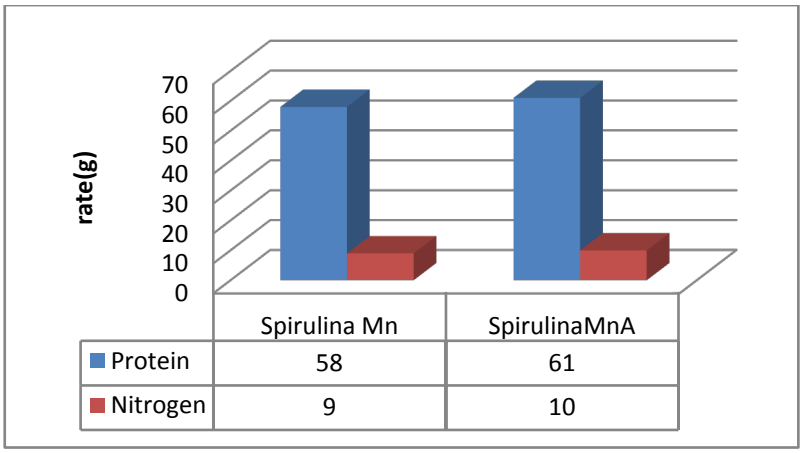
The rate of β- carotene in MnA is high compared to mn. Thus, the chlorophyl A rate and B are higher in MnA compared to mn.

But in the two cultures mn and MnA, the Xanthophyll rate does not vary

d. Rates of Proteins in 100g of Spirulina

The proteins remain a very abundant and very important element in the Composition of the components of Spirulina.

Elements(g)	*Spirulina Mn*	*SpirulinaMnA*
Protein	58	61
Nitrogen	9	10

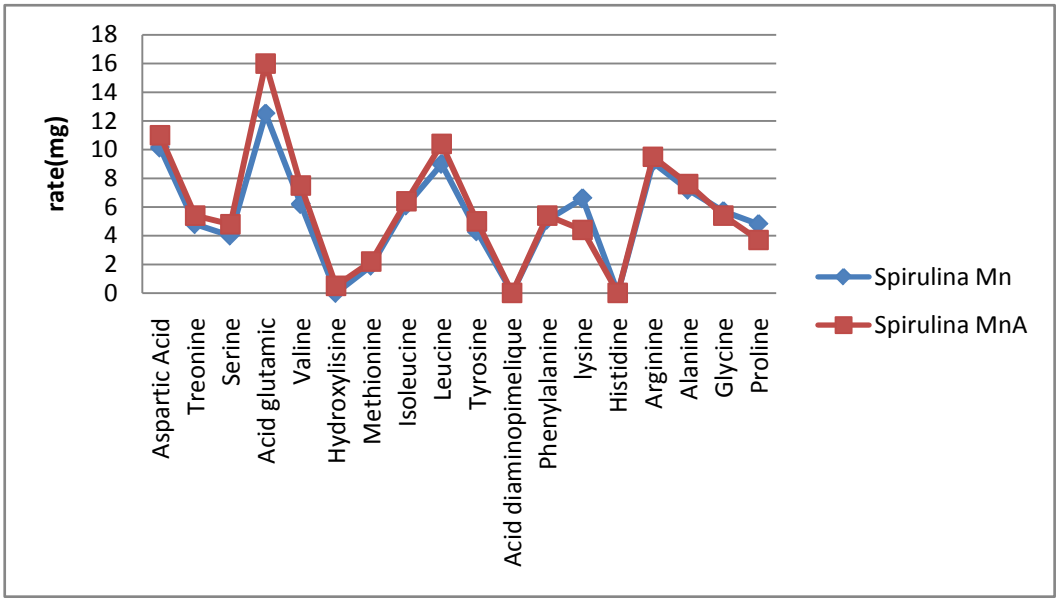


The protein rate varies according to the culture. But the other proteins which constitute some part of total proteins.

e. Composition in amino-acids of total proteins in 100g of Spirulina

In the result, one notices the presence of many essential amino-acids.

aminoacids (mg)	*Spirulina Mn*	*SpirulinaMnA*
aspartic Acid	10,14	11
Threonine	4,8	5,4
Serine	4	4,8
glutamic Acid	12,5	16,3
Valine	6,2	7,5
Hydroxylisine	0	0,5
Methionine	1,9	2,2
Isoleucine	6,1	6,4
Leucine	9,0	10,4
Tyrosine	4,3	5,0
diaminopimelic Acid	Trace	Trace
Phenylalanine	5,1	5,4
Lysine	6,6	4,4
Histidine	Trace	1,8
Arginine	9,1	9,5
Alanine	7,2	7,6
Glycine	5,7	5,4
Proline	4,8	3,7

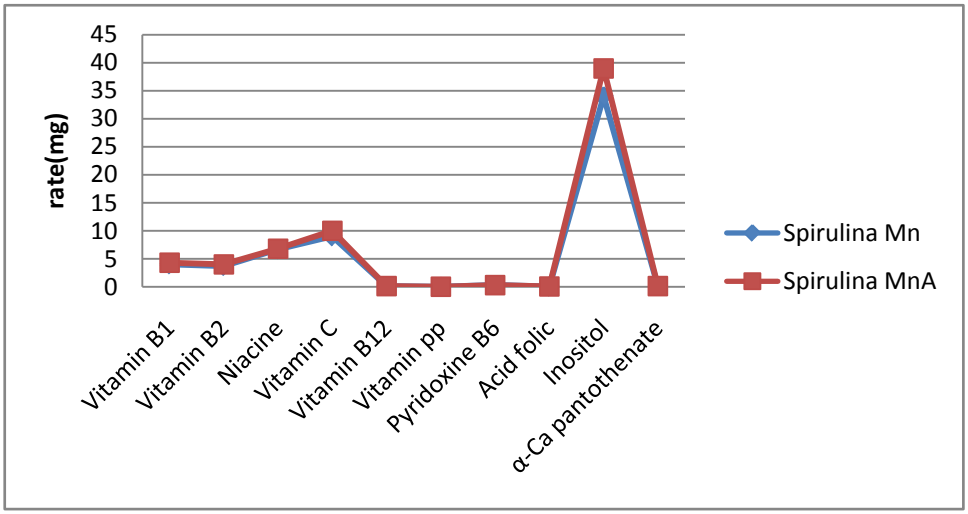


The rate various amino-acids in two stocks different coming from the two different cultivation vary from one cultivation to another. The presence of the essential acids justifies the protein importance in Spirulina. The living beings need the amino-acids especially the essential amino-acids. With the education level of the amino-acids in Spirulina, it appears that 99% of rate of amino-acids, present in the natural environment improve is higher compared to the natural environment.

f. Rates of Vitamins in 100 g of Spirulina

The table shows the interesting vitamins in Spirulina

Vitamin (mg)	Spirulina Mn	Spirulina MnA
Vitamin B1	4,00	43
Vitamin B2	3,7	4
Niacine	6,7	6,8
Vitamin C	9,0	10
Vitamin B12	0,12	0,13
Vitamin pp	Trace	Trace
Pyridoxin B6	0,32	0,30
folic Acid	0,034	0,056
Inositol	34	39
∝- Ca pantothenate	0,15	1,12

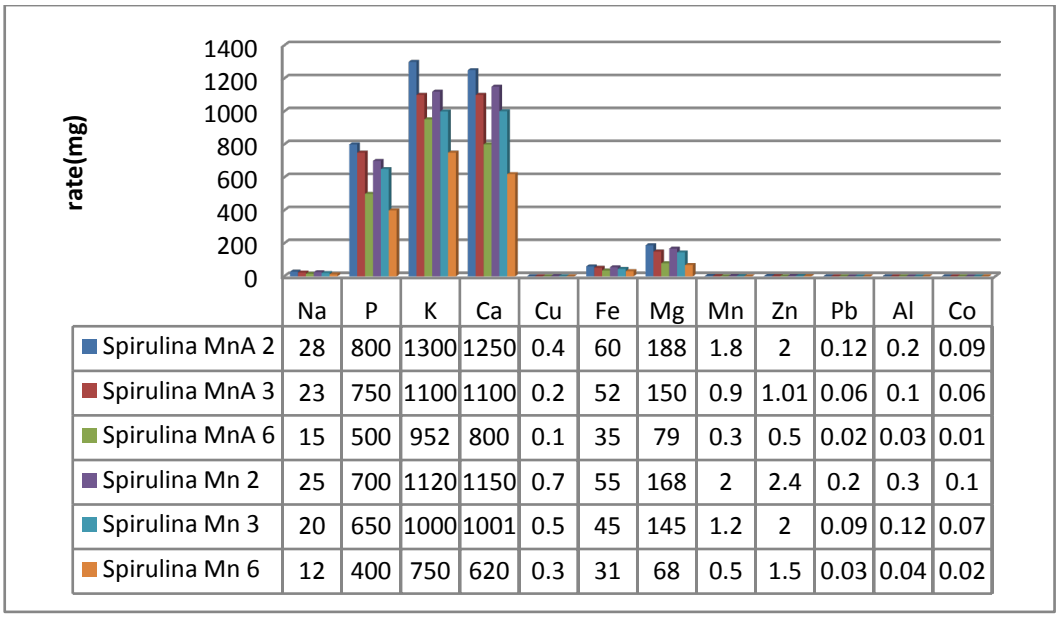


The vitamins in Spirulina remain with a very small rate or with the state of trace. The rate of vitamins varies one with the other. One notice in the stock coming from MnA, the rate of the vitamins are higher compared to Mn. That explains why the physicochemical parameters influence the formation of the vitamins in Spirulina.

B. Qualitative Analysis after the conservation

a) Rates of minerals in 100g of Spirulina

	Spirulina MnA			Spirulina Mn		
months / Minerals (mg)	2	3	6	2	3	6
Na	28	23	15	25	20	12
P	800	750	500	700	650	400
K	1300	1100	952	1120	1000	750
Ca	1250	1100	800	1150	1001	620
Cu	0,4	0,2	0,1	0,7	0,5	0,3
Fe	60	52	35	55	45	31
Mg	188	150	79	168	145	68
Mn	1,8	0,9	0,3	2	1,2	0,5
Zn	2	1,02	0,5	2,4	2	1,5
Pb	0,12	0,06	0,02	0,2	0,09	0,03
Al	0,2	0,1	0,03	0,3	0,12	0,4
Co	0,09	0,06	0,01	0,1	0,07	0,02

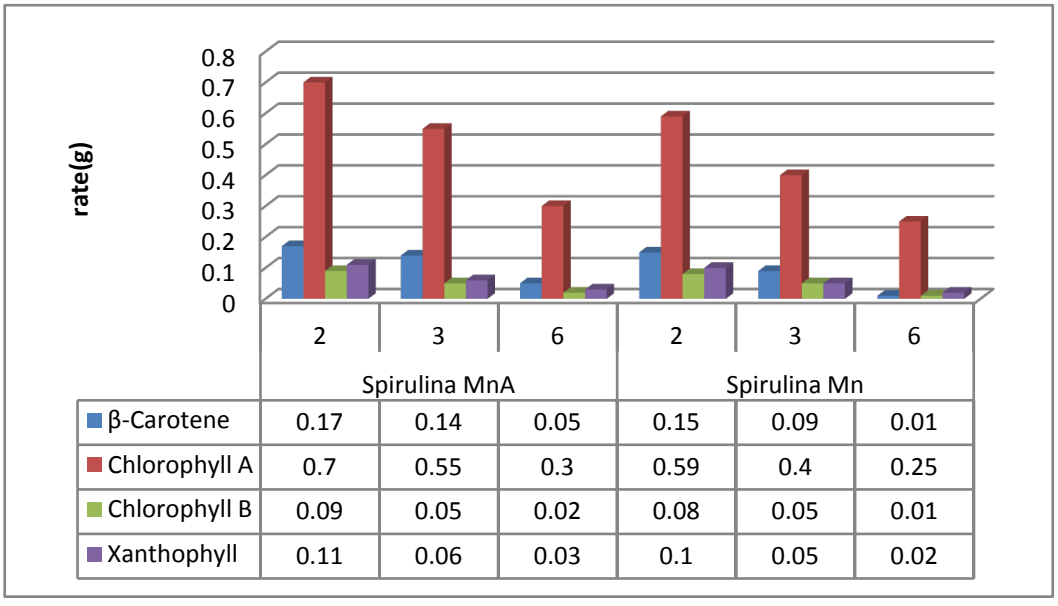


	Na	P	K	Ca	Cu	Fe	Mg	Mn	Zn	Pb	Al	Co
Spirulina MnA 2	28	800	1300	1250	0.4	60	188	1.8	2	0.12	0.2	0.09
Spirulina MnA 3	23	750	1100	1100	0.2	52	150	0.9	1.01	0.06	0.1	0.06
Spirulina MnA 6	15	500	952	800	0.1	35	79	0.3	0.5	0.02	0.03	0.01
Spirulina Mn 2	25	700	1120	1150	0.7	55	168	2	2.4	0.2	0.3	0.1
Spirulina Mn 3	20	650	1000	1001	0.5	45	145	1.2	2	0.09	0.12	0.07
Spirulina Mn 6	12	400	750	620	0.3	31	68	0.5	1.5	0.03	0.04	0.02

After the conservation, the rates of minerals decrease almost 3/4 and especially the minerals very volatile like Ca, Mg.

b) Rates of Pigments in 100g of Spirulina

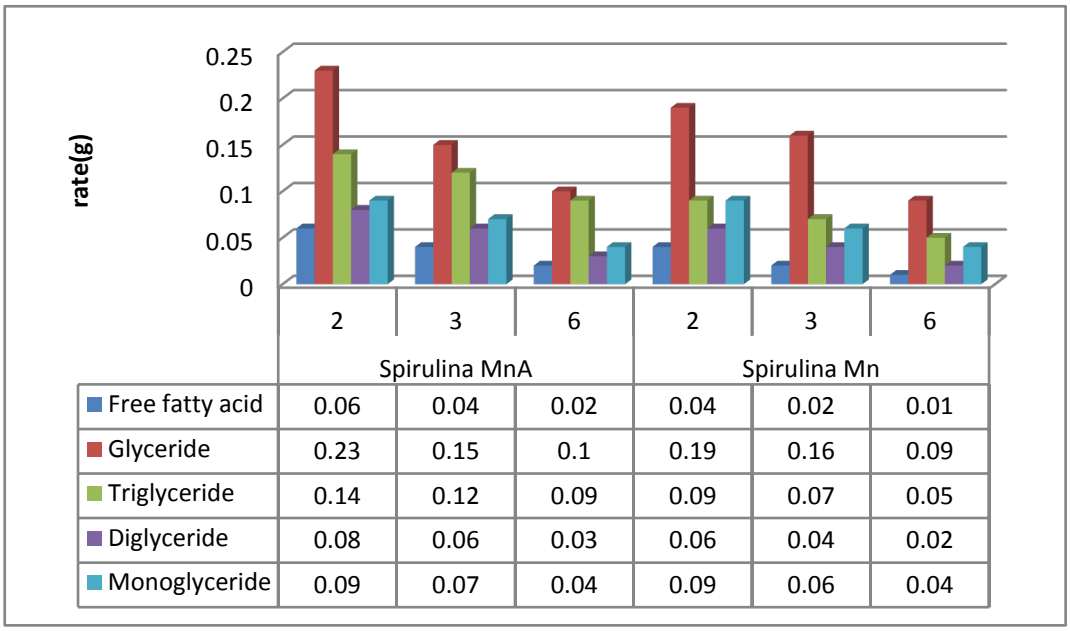
	Spirulina MnA			Spirulina Mn		
months / Pigments (g)	2	3	6	2	3	6
β-carotenoid	0,17	0,14	0,05	0,15	0,09	0,02
Chlorophylle A	0,70	0,55	0,30	0,59	0,40	0,25
Chlorophylle B	0,09	0,05	0,02	0,08	0,04	0,01
Xanthophylle	0,11	0,06	0,03	0,10	0,05	0,02



	2	3	6	2	3	6
	Spirulina MnA			Spirulina Mn		
β-Carotene	0.17	0.14	0.05	0.15	0.09	0.01
Chlorophyll A	0.7	0.55	0.3	0.59	0.4	0.25
Chlorophyll B	0.09	0.05	0.02	0.08	0.05	0.01
Xanthophyll	0.11	0.06	0.03	0.1	0.05	0.02

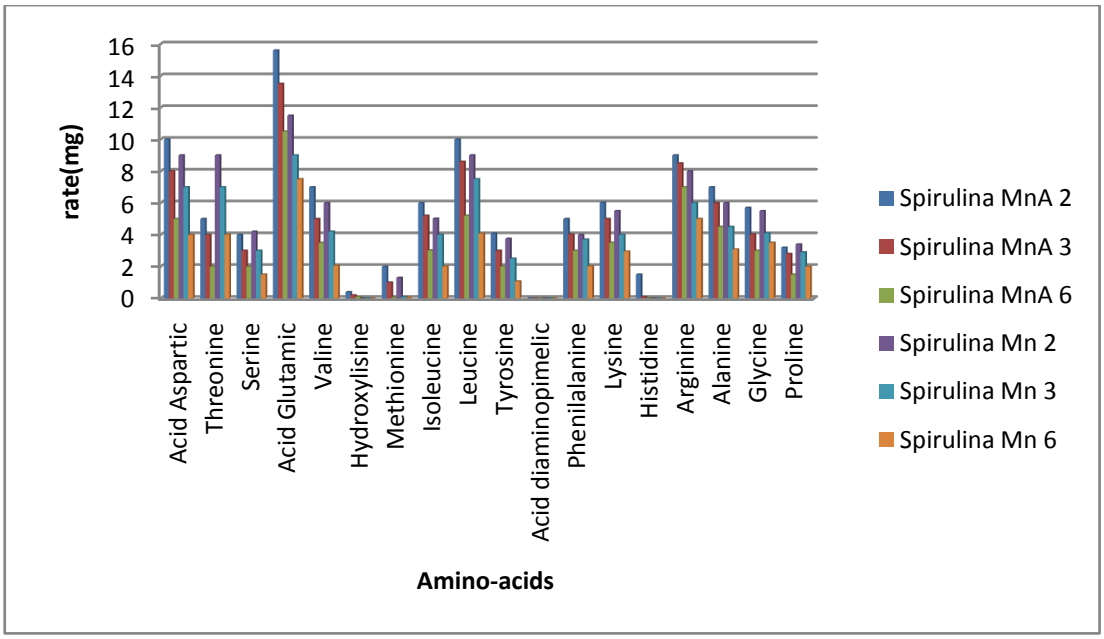
c. Rates of Lipids in 100g of Spirulina

	Spirulina MnA			Spirulina Mn		
Months / Lipids	2	3	6	2	3	6
Free fat acid	0,06	0,04	0,02	0,04	0,02	0,01
Glycerids	0,23	0,15	0,10	0,19	0,16	0,09
Triglycerids	0,14	0,12	0,09	0,09	0,07	0,05
Diglycerids	0,08	0,06	0,03	0,06	0,04	0,02
Monoglycerids	0,09	0,07	0,04	0,09	0,06	0,04



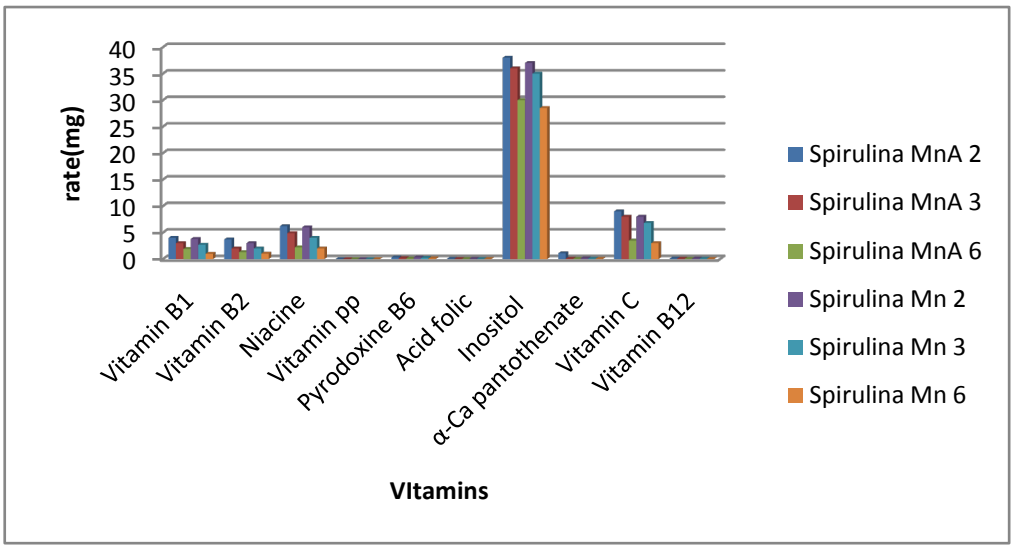
d) Rates of amino-acids in 100g of Spirulina

	Spirulina MnA			Spirulina Mn		
months / amino Acid (g)	2	3	6	2	3	6
aspartic Acid	10	8	5	9	7	4
Threonin	5	4	2	4,2	3	1,5
Serin	4	3	1,9	3,5	2,2	1
glutamic Acid	15,6	13,5	10,5	11,5	9	7,5
Valin	7	5	3,5	6	4,2	2,06
Hydroxylisin	0,4	0,2	0,09	0	0	0
Methionin	2	1	0,07	1,3	0,09	0,05
Isoleucin	6	5,2	3,02	5,02	4	2,01
Leucin	10	8,60	5,2	9	7,5	4,08
Tyrosin	4,09	3	2,01	3,75	2,5	1,07
diaminopimelic Acid	0,001	0	0	0	0	0
Phenylalanin	5	4,02	3	4	3,7	2,02
Lysin	6,02	5	3,5	5,5	4	2,99
Histidin	1,5	0,09	0,02	0,001	0	0
Arginin	9	8,5	7	8	6	5
Alanin	7	6	4,5	6	4,5	3,09
Glycin	5,2	4,03	3	5,5	4,1	3,5
Prolin	3,2	2,8	1,5	3,4	2,9	2



e) Rates of Vitamins in 100g of Spirulina

	Spirulina MnA			Spirulina Mn		
Months / Vitamins(mg)	2	3	6	2	3	6
Vitamin B1	4	3	1,9	3,8	2,7	1
Vitamin B2	3,7	2,01	1,3	3	2	1,03
Niacine	6,2	4,9	2,2	6	4	2
Vitamine pp	0,001	0	0	0,001	0	0
Pyridoxine B6	0,29	0,18	0,10	0,30	0,22	0,13
folic Acid	0,05	0,03	0,01	0,04	0,02	0,009
Inositol	38	36	30	37	35	28,5
α-Ca pantothenate	1,10	0,09	0,05	0,15	0,08	0,04
Vitamin C	9	8	3,5	8	6,8	3
Vitamin B12	0,11	0,08	0,04	0,10	0,075	0,03



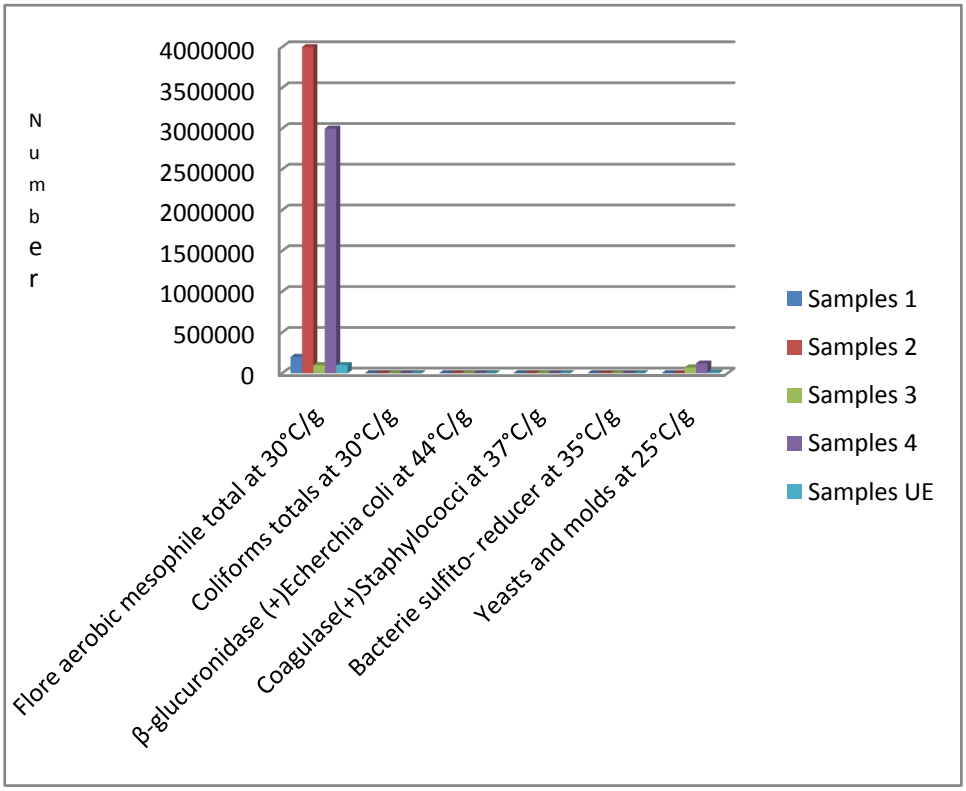
C- Microbiological analysis after ten days of conservation

The test of microbiological is done in two different cultivation microbiological

a) Quality in the natural environment (Mn)

The criteria are referred in the dehydrated crop products. The presence of aerobic flora mesophilic total in Spirulina marks the presence of many micro-organisms. In other case the presence of Staphilococcus marks the presence of toxin. The Sulfitoreducer bacteria are very abundant in Spirulina cultivated in the natural environments. This presence marks the contamination by faeces and the hygienic quality of adopted technology is not clean.

Samples	01	02	03	04	UE standard
All Aerobic mesophilic Flora at 30°C/g	2.10^5	4.10^6	10^5	3.10^6	10^5
All Coliforms at 30° C / g	Ne = 10	Ne = 11	Ne = 15	Ne = 10	100
Escherichia coli β-glucuronidase (+) at 44°C/g	11	12	10	13	10
Staphylococcus coagulases (+) at 37°C/g	111	97	112	100	100
Bacteries sulfitoreducer at 37°C/g	$1,4.10^3$	$1,4.10^3$	7.10^3	$1,4.10^3$	10^4
Yeasts and moulds at 25°C/g	9.10^3	10.10^3	7.10^3	12.10^3	10^4
Salmonella at 25°C/g	Absence	Absence	Absence	Absence	Absence
Conclusion per sample	unsatisfied	unsatisfied	unsatisfied	unsatisfied	



According to the microbiological analysis, the 4 analyzed samples of Spirulina are nonsatisfactory.

b) Microbiological quality in the improved natural environment (MnA)

The rate of aerobic flora mesophilic total in the improved natural environments is about equal to the limit of standard EU. The rate varies between 2.104 to 8.104. We note that majority of aerobic flora mesophilic total is composed by halophile germs.

In the pathogenic micro-organisms Coliforms totals and Staphilococca coagulases are lower than 100. With regard to the Bacteria sulfitoreducer, the rate is limited to the standard

of EU between 8 to 10. They are in proportion high and are likely to produce toxins. But the presence of bacteria sulfitoreducer, in Spirulina put in question because Spirulina sharp in a high salinity and constitutes nitrite which are used as sources of nitrogen. Thus the bacteria are non-existent in the culture of development and can be brought outside.

According to the two tables giving the microbiologic quality of Spirulina, the presence or not of micro-organism pathogenic depends on the microbiological quality of the water of washing. But for the sulfitoreducer which are not present in the culture media, the operation of drying is rather responsible for the post contamination because it is carried out in a traditional way on trays of the free air. The products are thus exposed to all the vectors of contamination. And as these germs are the telluric ones i.e. live in the ground and of the table companions of the intestine thus are in the feces, they can thus be brought by dust, the winds, the insects, equipment and the manipulators.

6. Discussion

Spirulina is a very complete food, but one some remark on the level of the two different cultivation. The rates of heavy metals are increased in the cultivation of Spirulina Mn compared to the Spirulina MnA. Therefore, the difference comes from sea water and is in hiding. Even of another element like the pigments, the amino-acids, the lipids and the protein are important in the natural cultivation compared to the improved natural cultivation. The microbiological analysis show that the number of bacteria in the improved natural environment is satisfactory compared to the standard of European Union. On the level of conservation, Spirulina loses almost 3/4 of value of the components after 6 months of conservation. The fall of this rate comes from mode of conservation and preparation. Therefore, for the cultivation, the natural environment is advised for the interested party with the cultivation of Spirulina. Our study is made in the North of Madagascar, therefore the results are very satisfactory that it is on the level of the physicochemical parameters, that it is on the level biochemical parameters.

7. Conclusions

The area of Sambirano is very favorable to the cultivation of Spirulina. The climates, salinity, the pH, the structure of the ground allow the cultivation of Spirulina. The results show that the rates of the components are not differing of those from the South of Madagascar. Therefore, to ensure and stabilize the components, it is necessary to set up the method HACCP and the strict method of conservation.

REFERENCES

[1] Alava V., R. and Lim C.,1983. The quantitative dietary protein requirement of Penaeus monodon juveniles in a controlled environment, aquaculture; 30,53.

[2] Afnor,1995. Recherche et sélection de descripteurs pour l'élaboration d'un profile sensoriel, par approche multi dimensionnelle 1ère édition. Afnor. Paris ; p : 1, 25.

[3] Afnor, 1994. Microbiologique alimentaire. Méthode de routine pour le dénombrement de Staphylocoque à coagulase positive par comptage des colonies à 57°C. NFV 08-057 AFNOR, p : 419-433.

[4] Afnor, 1984. Microbiologique alimentaire directives générales pour les examens pour le dénombrement des Staphylococcus aureus méthode par comptage des colonies. NFV 08-014, 150 6888, Afnor, p : 113-120.

[5] Afnor, 2001. microbiologie des aliments. Méthode horizontale pour le dénombrement des Escherichia coli glucuronidase positive par comptage des colonies à 44°C, NF ISO 16649-2, Afnor : p : 1-8.

[6] Arpin M. et Col., 1969. Méthodes modernes d'analyse structurale des caroténoïdes. Produits Probl. pharm., 24: 630,44.

[7] Belay A, 2008. Spirulina (Arthrospira) production and quality assurance. In: Spirulina in Human Nutrition and Health. (Eds. Gershwin, E. and Belay, A.), CRC press, Taylor & France Group, Boca Raton, London, New York, pp. 1-23.

[8] Busson F,1971. Spirulina plantensis (Gni) Ceîtter et Spirulina geîtteri J de Toni, Cyanophycées alimentaires. Marseille: Armée français, service de santé, Parc Pharo.

[9] Camille V. et coll, 2010. Comment évaluer la qualité gustative d'un produit; 6,10.

[10] Cazal B, 2010. La revue de OSO : Aquaculture Bio; 1, 2.

[11] Ciferri O, 1983. Spirulina, The edible micro-organism. Journal of Microbiology; 47(4): 551-578.

[12] Clement G.,1975. Production et constituants caractéristique des algues Spirulina plantesis et maxima. Annales de la nutrition et de l'alimentation, 29,6.

[13] Cozzone A., Busson F., 1970. Electrophorèse en gel de Polyacrylamide des protéines de S. plantesis et de S. gitleri.C.R.hebd. Séanc-Acad SC. Paris.

[14] Dillon C. and Phan A.,1993. Spirulina as a source of proteins in human nutrition. Bull. Inst. Océano, Monaco, 12: 103-107.

[15] Davies B.H., 1965. Chemistry and Biochemistry of plant pigmants . Op. Cit..

[16] Edis KORU., 2012. Earth Food Spirulina (Arthrospira): Production and Quality Standards, Food additive. Prof. Yehia El-Samragy(Ed, ISBN:978-953-51-0067-6.

[17] Fanjanarivo S., 1999. Production de Spirulina au Stade d'essai dans le média de demain, , 27-28-29.

[18] Fevrier C., 1973. Recent developments in Spirulina, Report of the Third meeting of the PAG AD HOC. Working group on single cells Protein, USA; p.14.

[19] Fox R.,1986. L'algoculture : La spirulina, un espoir dans le monde de la faim. Aix – en Province : EDISUD.

[20] Henri R., Manasori S., 2000. Rapport technique, 15; 25, 215.

[21] Huang Y.S., 1988. Growth effect of Penaeus monodon fed with different combinaisons of dry heat toasted brown rice and wheat flour diet, J. Fish. Soc. Taiwan ;15,21.

[22] Japan Food, 1977. Desearxch Laboratory, Analysis Report.

[23] Johnson P. and Shubert, E., 1986. Availability of iron to rats from Spirulina, a blue green algae. Nutr. Res., 6: 85-94.

[24] Kanazawa A., Tanako N. and Teshima S, 1971.. Nutritional requirement of prawn II. Requirement for sterol s, bull. Jpn. Soc. Sci. Fish; 33,211.

[25] Kayama M., Hirata M., Kanazawa A., Tokiwas., and Saito M., 1980. Essential fatty acids in the diet of praw III. Lipid metabolism fatty acid composition, bull. Jpn. Soc. Sci. Fish.; 46; 483.

[26] Krauss B. H., 1979. Native Plants Used as Medecine in Howaï.

[27] Lan C.C.,1991. Proteases distribution in Crass Prawn (Penaeus monodon) digestive Tract and Thein Effects on Feed Digestibility, Ph.D. Thesis, National Taiwan Océane University, keeling, Taiwan, R.OC; 191.

[28] Lee D.L., 1971. Studies on the protein utilization relate to growth of Penaeus monodon fabriius , aquaculture;1;1 .

[29] Missouri Botanical Gardeau, 2006. Angiosperm Phylogeny.

[30] Penaflorida V.D., 1989. An evaluation of indigenous protein sources as potential component in the diet formulation for tiger Prawan ,Pénaeus monodon, using essential amino acid index (EAAI) Aquaculture;83;319.

[31] Parvin M., 2006. Culture and growth performance of Spirulina platensis in supernatant of digested poultry waste. Bangladesh Agricultural University, Mymensingh, Bangladesh. (M.S. Thesis) .

[32] Rasamoelina H.,1999. La spirulina de Madagascar valeur nutritionnelle et qualité microbiologique. Mémoire de fin d'étude, Département I.A.A./ E.S.S.A.

[33] Rabodomalala Z., Suemitsu M.,2001. le projet de développement de l'aquaculture. Rapport technique, 21.200.

[34] Richard D., Sunghee L., Peeggy C.F., Hazel F., 1965. Utilization of algae as a protein source for humans. J .Nutr .;86,376.

[35] Sheen S.S. and Chen J.C., 1991. The feasibility of extruded rice in Shrimp feed to replace wheat floor for tiger prawn Penaeus monodon, in abstract: 22nd Annual Meeting of the World Aquaculture society, world Aquaculture society Baton Rouge, LA.

[36] Sano M., 1999. Microbiologie de base de l'agriculture marine des crevettes.

[37] SauvageotF., 1990. L'évaluation des denrées alimentaires aspect méthodologique. Lavoisier. Tee et doc. Paris, 195.

[38] Shiau S.Y., Kwork C.C, and Chon B.S., 1991. Option dietary protein level of Penaeus monodon reared in seawater and brackish water, Nippoon suisan Gakkaishi;57;711.

[39] Wagner W.L.,Herbert D.L, and Sohmer S.H.,1990. Manual of the flowering Plants of Hawaï.

[40] Wang Z. P.,2005. Morphological reversion of Spirulina (Arthrospira platensis) (cyanophyta): from linear to helical. Journal of Phycology ;41(3) : 622-628.

[41] Belay, A. (2008). Spirulina (Arthrospira) production and quality assurance. In: Spirulina in Human Nutrition and Health. (Eds. Gershwin, E. and Belay, A.), CRC press, Taylor & France Group, Boca Raton, London, New York, pp. 1-23.