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TOTAL PROTEIN PROPORTIONS DETERMINATION IN SPIRULINA PRODUCED IN BURKINA FASO

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ABSTRACT

Spirulina is a food supplement rich in micronutrients and vitamins. It is called "the ideal food for humanity" and the World Health Organization has considered it a "super food" and the best food for the future due to its high nutritional value. The present study aimed to determine the proportions of total proteins in sun-dried spirulina. This was an analytical titration study using the KJELDAHL method. The average proportion of proteins in the spirulina samples was 47.52%. The lowest proportion was 43.14% and the highest was 56.10%. There is no significant difference between the variations in proportions of total proteins of the samples studied. However, the protein proportions of spirulina depend considerably on the climate and the growing area. Drying and packaging spirulina remains the only means of wide commercial distribution. Given the nutritional losses that can occur during storage, it is suggested that spirulina be consumed quickly after harvesting in order to maximize the nutrient benefit.

Keywords: Burkina Faso, Spirulina, Total proteins.

1. Introduction

In the plant environment, spirulina represents more than 18% and has around 20,000 species worldwide (Lecointre et al.; 2001). Indeed, under the term "algae" are grouped plant organisms that are extremely varied both in size and cellular structure. Spirulina (*Spirulina platensis*, family of Oscillatoriaceae) is one of the blue-green algae which is rich in protein (62.84%) and contains a high proportion of essential amino acids (38.46% of protein) and is rich in vitamins such as β-carotenes and vitamin B complex in the form of vitamin B12 (175 µg/100g) and folic acid (9.92 mg/100 g). It is also rich in calcium and iron (922.28 and 273.2 mg/100g, respectively) to protect against osteoporosis and blood diseases, as well as a high percentage of natural fiber (Sharoba, 2014).

Thus, spirulina is useful and necessary for the infant's growth and very suitable for children, especially in the growth phase, the elderly and the visually impaired. It also helps a lot in general weakness, anemia and chronic constipation. Spirulina contains selenium (0.0393 mg/100g) and many phyto-pigments such as chlorophyll and phycocyanin (1.56 and 14.647%), which are known to be powerful antioxidants (Sharoba, 2014).

Spirulina has been proven experimentally, in vivo and in vitro to be effective in treating allergies, anemia, cancer, hepatonephrotoxicity, viral and cardiovascular diseases, hyperglycemia, hyperlipidemia, immunodeficiency and inflammatory processes, among others (Chamorro G et al., 2002, Romay Ch et al., 2003, Selmi C et al., 2011, Abdel-Daim M.M et al., 2015). Thus, spirulina is generally considered to be a product without toxicological effects, and it is approved by the FDA (Food and Drug Administration) and ANVISA (Navacchi M.F.P et al., 2012). Formulators use spirulina in specialty food bars, powdered nutritional drinks, beverages, fruits and juices, frozen desserts and condiments (Sharoba, 2014). Over the last decade, agriculture and consumption of this algae are booming in all developing countries (Charpy L et al., 2008). Its consumption is indicated as one of the solutions to fight multi-nutrient deficiencies in these countries (Simporé J et al., 2007, Teas J et al., 2012).

This food supplement offers remarkable health benefits for undernourished children. It is rich in β-carotene which can overcome eye problems caused by vitamin A deficiency; it provides the daily dietary requirement of β-carotene which can help prevent blindness and eye diseases (Seshadri.,1993). The protein and vitamin B complex provides a major nutritional improvement in an infant's diet. It is the only food source other than breast milk containing substantial amounts of essential fatty acid, essential amino acids which helps regulate the entire hormonal system (Ramesh S et al., 2013). Spirulina also contains a-tocopherol which is known in most cases for its anti-oxidant properties and its involvement in the regulation of oxidative stress (Falquet J et al., 2006, McLaren SD et al., 2001, Traber M.G et al., 2007, Rajendran B et al., 2013).

Therefore, spirulina constitutes an interesting source of β-carotene and a-tocopherol which both need to be controlled during production and processing. In Burkina Faso, the deficiency of several nutrients is a public health problem. One of the solutions undertaken in the fight against nutritional deficiencies is to introduce the spirulina cultivation and the popularization of its consumption in small doses as a food supplement (Sawadogo M et al., 2004). The main objective of farms involved in spirulina production is to contribute to improving the health of the community through the use of spirulina (Sawadogo M et al., 2004). The government of Burkina Faso has approved this objective by fully financing the Koudougou "Nayalgué" integrated spirulina production project.

Fresh spirulina remains less accessible to the population due to its short shelf life. Drying fresh biomass and storing it remains the only safe means of marketing or humanitarian distribution. Drying allows stabilization of the hydrated spirulina by reducing both the water content. However, chemical changes are likely to occur in dry biomass during drying and storage. Oxidizing components such as α-tocopherol and β-carotene are sensitive to atmospheric oxygen, light and temperature variations (Rastrelli L et al., 2002, Cuvelier C et al., 2003, Ferreira J.E et al., 2008). The aim of the study is to evaluate the total protein proportions of spirulina samples produced in Burkina Faso after drying.

2. Material and methods

2.1 Plant material

The plant material consisted of spirulina (*Spirulina platensis*) collected from may to june 2020 from the « Notre Dame d'Afrique production » farm in Koudougou (Burkina Faso). Spirulina was obtained in controlled synthetic culture for production on a semi-industrial scale.

2.2 Sampling

A sample of Thirty (30) sachets of spirulina powder produced on the farm was randomly chosen for chemical analyzes in the laboratory to determine total protein levels.

2.3 Drying spirulina

Drying was carried out in the sun for 5 hours using a "shell" type dryer (Ibrahim E.A et al., 2015). After drying the spirulina spaghetti, they became hard and brittle, easy to grind. After drying, all samples were removed from the dryer and placed in an aluminum plate. The dried biomass was immediately weighed and crushed. Powder conditioning was carried out in a room with closed windows at room temperature (30 °C). The powder was manually packaged in plastic bags (10 g/bag). The powder samples were immediately transported in an insulated bag to the health sciences research institute laboratory of where chemical analyzes of the nutrients were carried out. The 30 powder samples were analyzed upon arrival to determine total protein levels. Total proteins were measured by the KJELDAHL titrimetry method.

2.4 Methods for measuring protein in spirulina

2.4.1 Principle

The oxidizing action of boiling concentrated sulfuric acid transforms organic nitrogen into mineral nitrogen in the form of ammonium sulfate $(NH4)2SO4$. After displacement by soda, the ammonia is distilled and collected in a boric acid solution which traps it. This colored solution is then titrated by acidimetry.

2.4.2 Procedure

A mass of 0.1 gram of the sample is introduced into a vial to which 4 ml of concentrated sulfuric acid (36 N) is added. The device temperature is then set to 440°C, then the vial containing the sample is placed on the hot plate. After four minutes, 5 ml of hydrogen peroxide is added to the carbonized sample through the funnel of the fractionation column. The vial is then removed from the hot plate and the mineralized material is diluted after cooling with water to the mark. The diluted mineralized material is transferred to a distillation flask by adding another 100 ml of water, a 10 N sodium hydroxide solution in the presence of phenolphthalein until a pink color is obtained. This solution is then distilled and the distillate is trapped in 20 ml of boric acid contained in a 250 ml flask. The distillation is stopped when the final volume reaches 150 ml. The distillate is then titrated with 0.1 N sulfuric acid. The end of the reaction is marked by changing color. A blank is made using the same procedure.

2.4.3 Expression of results

The following formula makes it possible to determine the percentage of total nitrogen and by conversion the total protein levels.

$$\text{Total protein levels (\%)} = \frac{VE - VB}{PE} \times N \times 14\times10^{-3} \times 6,25 \times 100$$

PE: test portion (0.1 g)
VE: volume of sulfuric acid necessary to titrate the sample
VB: volume of sulfuric acid necessary to titrate the blank.
N: Sulfuric acid Titration (0.1 N)
6.25: conversion factor
10^{-3}: dilution factor
14: Nitrogen atomic mass

3. Results

The total protein proportions was carried out on 30 samples after drying. The results are recorded in the table below.

Table 1 : Proportions of total proteins in spirulina samples after drying

Sample Number	Total Protein averages in % ± standard deviation	
1	47,64	0,55
2	45,46	1,49
3	52,39	0,56
4	48,78	1,30
5	49,46	2,31
6	51,67	1,41
7	48,47	1,43
8	53,20	0,51
9	48,03	1,81
10	56,01	0,31
11	49,38	1,73
12	52,49	1,34

13	49,95	1,02
14	49,47	0,71
15	56,69	0,22
16	44,82	0,91
17	45,72	0,46
18	47,60	0,83
19	46,57	0,93
20	45,78	0,37
21	50,73	1,81
22	44,50	2,19
23	43,14	1,89
24	56,10	1,11
25	47,55	0,64
26	47,85	1,16
27	48,91	1,14
28	46,58	1,43
29	46,74	1,02
30	49,97	1,10
Average	47,52	0,67

The Table 1 presents the proportions of total proteins in 30 spirulina samples after drying. Overall, there is an average of 47.52% in total protein. The results presented constitute the mean ± standard deviations (each sample was analyzed in duplicate and the mean was calculated). The lowest average is 43.14% and the highest is 56.10%. Fisher's exact test shows that there is no significant difference between the protein contents of the spirulina samples analyzed (P > 0.3).

The Figure 1 shows the protein proportions histogram of the 30 spirulina samples. Overall, there is a slight variation between the proportions but this difference is statistically insignificant.

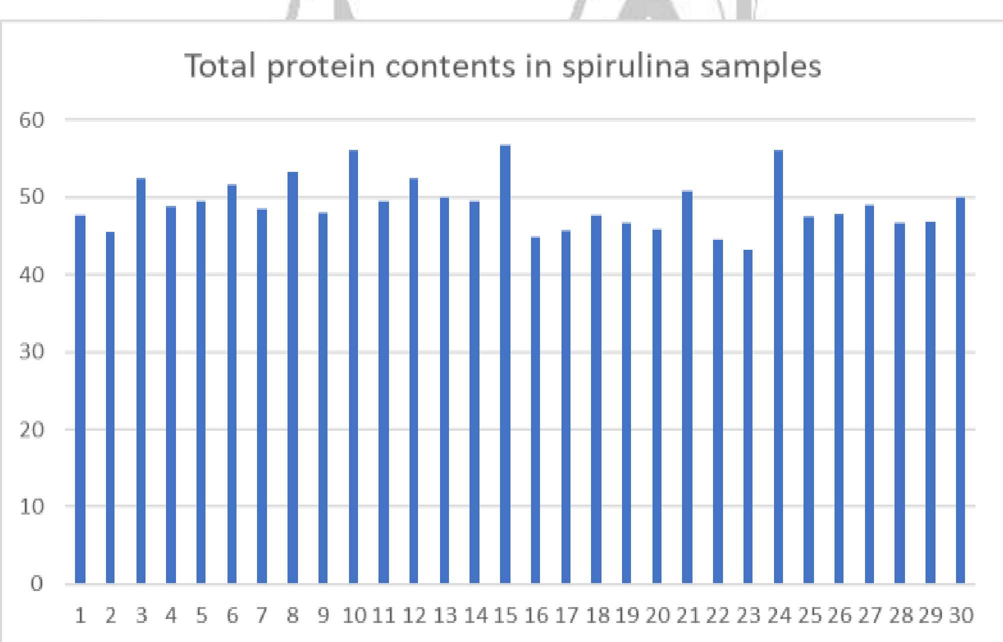

Figure 1 : Histogram of protein proportions in spirulina samples
X= sample number (from 1 to 30)
Y= Protein proportions of spirulina samples (in %)

4. Discussion

We got an average of 47.52% protein. It appears from these results that spirulina is indeed a food very rich in proteins, even though these values are below those obtained by Dansou in 2003 (57.10%), Kabore in 2001 (65%), Falquet in 2006 (50-70%) and Clement in 1975 (72%) (Falquet J et al., 2006). These figures acquire greater significance when compared with the average protein contents of other vegetables seeds which, like cyanophyceae, are nitrogen fixers: beans (22%) and even soya (38%), although renowned for its rich protein content. In our developing countries, protein intake of animal origin is expensive and difficult to handle. Indeed, the main protein sources of animal origin are perishable foodstuffs which require special storage conditions. In this context, spirulina could balance protein-deficient diets, especially in children because its handling is less restrictive. Based on this observation, spirulina could enter nutritional recovery programs if its cost offers a real advantage. Unlike other protein sources, spirulina does not contain cellulosic walls but a relatively fragile envelope. This fact explains the very good proteins digestibility in simply dried spirulina. Spirulina therefore requires neither cooking nor special treatments intended to make its proteins accessible. This is a considerable advantage for the high value constituents preservation such as vitamins and polyunsaturated fatty acids.

Studies undertaken by Kambou et al. in 2005 on spirulina in Burkina Faso, showed an average protein value of 47.87 (Kambou C., 2005). These results are similar to those found in the present study (47.52%). Falquet et al. in 2006 found that proteins represented 50 to 70% of the total weight of spirulina. A UNESCO report indicates that spirulina cultivated in Lake Chad in 2023 showed protein proportions between 60 and 70%.

The protein content therefore varies depending on the growing area. These disparities in results could come from several factors depending primarily on the growing conditions, the climate but especially the drying of the spirulina. It is, for example, very likely that drying by "spray-drying" which very strongly breaks the spirulina filaments will considerably reduce the proteins shelf life.

The technique used in the present study was direct sun drying. With this in mind, fresh biomass was transformed into cylinders two millimeters in diameter (spirulina "spaghetti"), then spread on a screen contained in a drying box exposed to sunlight. The air entering temperature of the drying box, which turns out to be a key parameter for guaranteeing the spirulina nutritional value, was not controlled. Samples dried using this technique contain non-homogeneous residual water contents which are likely to have an influence on the nutrient content (Bationo F et al., 2015).

Authors used spray drying where the juice of crushed spirulina was dried. In this regard, the filaments were previously pulped to break their membrane before being subjected to a stream of combustion gases at very high temperature for a very short time. Our samples would probably contain more residual water than those of these authors; which could explain the protein contents below 50% in the spirulina analyzed for the present study.

5. Conclusion

The results of the present study suggest the use of this micro-algae as a source of proteins in the fresh state or just after drying if we want to benefit appropriately from the the nutrients effects. Spirulina has a total proteins interesting proportion just after drying. However, previous studies have shown that prolonged storage of spirulina could cause losses of nutrients including proteins. It is therefore advisable to consume it immediately or shortly after its production if we want to maximize the nutritional benefit that this micro-algae provides.

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