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VARIATION IN SOME NUTRITIONAL QUALITIES (PROTEINS, AMINO ACIDS, PHYCOCYANIN) OF *SPIRULINA PLATENSIS* (CYANOBACTERIA) AS INFLUENCED BY SAMPLING SITES IN CHAD

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Abstract

This study was conducted in Chad to reveal changes in the nutritional composition of *Spirulina platensis* from different sampling sites. For each assessed parameter, the experimental design was a complete randomized block in which the sampling sites were considered as treatments, each one of which was replicated three times. As the outcomes of this research, samples from Kwa, CST1 and Artomissi sites displayed the greatest total protein concentrations with 64.26, 60.35 and 58.61 g/100g dry weight of Spirulina respectively. Madjorio site was the poorest of all amino acids assessed. Essential amino acids were present at variable concentrations, the highest accounting for Spirulina was from Kwa site. Tryptophan and cysteine were absent in all samples, while glutamic acid was the most represented amino acid in all samples with 10.78 g/100g dry weight of protein in sample from Kwa site. Histidine was the less concentrated amino acid (0.24 g/100g dry weight) in sample from Madjorio site. Phycocyanin was present in samples with concentrations ranging from 6.6-34.02 mg/100 g dry weight of Spirulina at Madjorio and Kwa sites. These findings reveal the nutritional value of this important microalgae, thus suggesting its use as dietary food supplement.

Keys words: *Spirulina platensis,* Amino acids, Total proteins, Phycocyanin, Chad.

Introduction

Spirulina platensis, a blue-green microalgae, is considered of having high nutritional quality due to its high digestibility and protein content, mainly in phycocyanin (Soronval, 1993). Protein content in *Spirulina platensis* was reported between 60-70% with most of the vital amino acids (Fox, 1999). About 15 g of protein that can be provided by Spirulina represents about 1/3 of daily needs in proteins for an individual of 60 kg (Briend, 1998). Therefore, for a child suffering from malnutrition, it would be realistic to add around 10 g of Spirulina in his daily food intake. This may represent according to child's weight, over 50% of the recommended protein contribution (Sguera, 2008). Considering this *Spirulina platensis* diet, it is unacceptable that over 800 million people in the world are still suffering from malnutrition, of which 200 million are children (Anon., 2006). Nowadays, malnutrition has become a serious problem of public health in many African countries where it causes over 30% of hospitalization with 10 to 20% mortality (Sall *et al.*, 1999). Regions where the demographic boom is higher are those where there is high demand for foods. In developing countries, malnutrition increases mortality risk, decreases immune defense, delays growth, and reduces learning and cognitive ability of children (Kapsiostis, 1967). Different organizations have chosen to help in preventing child malnutrition, through nutritional education of the population, development and consumption of locally grown products and the availability of additional food (Busson, 1971). Hence, many micro-seaweeds, particularly *Chlorella*, *Scenedesmus* and *Spirulina platensis* have drawn the attention of researchers as a source of proteins (Khilberg, 1972). Recent works on *Spirulina platensis* and other microorganisms widely used by men as additional food diet have highlighted their richness in several nutrients (Falquet & Hurni, 2006; Gerswhin & Belay, 2007). Also known as A*rthrospira fusiformis* (Voronnich), *Spirulina platensis* is a cyanobacteria dominating most of the flora of alkaline and saline polluted water (pH>11) that is responsible for reduced growth of other algae species (Castenholz *et al.*, 2001). Before this work, the only available data on nutritional quality of *Spirulina platensis* are those of organoleptic quality (Abdulqader *et al*., 2000; Sorto *et al.*, 2006; Yacoub, 2005), proteins content (Gongnet *et al.*, 2001; Mbaïguinam *et al*., 2006; Anon., 2009), some oligo-elements and heavy metal contents (Ngakou *et al*., 2012; Vicat *et al*., 2014). The aim of this study was to contribute to fighting against malnutrition by providing additional data on the nutritional characterization of *Spirulina platensis*. The awaited results could motivate the consumption of this single cell protein which nutritive values have been neglected in some African countries.

Materials and Methods

Description of the study area: The study was carried out in the sahelian zone (Fig. 1) comprising natural production sites of Lac Chad (Artomissi, Brandi, Kiri and Kwa) and those of Kanem (Touffou, Karga and Amerone). These two regions are located at between 300-350 km from the Nort-west of N'Djamena. The artificial production sites were those of Madjorio and of CST (Chad Sugar Company). The biological material used in the artificial culture medium was *Spirulina platensis* from the artificial CST site.

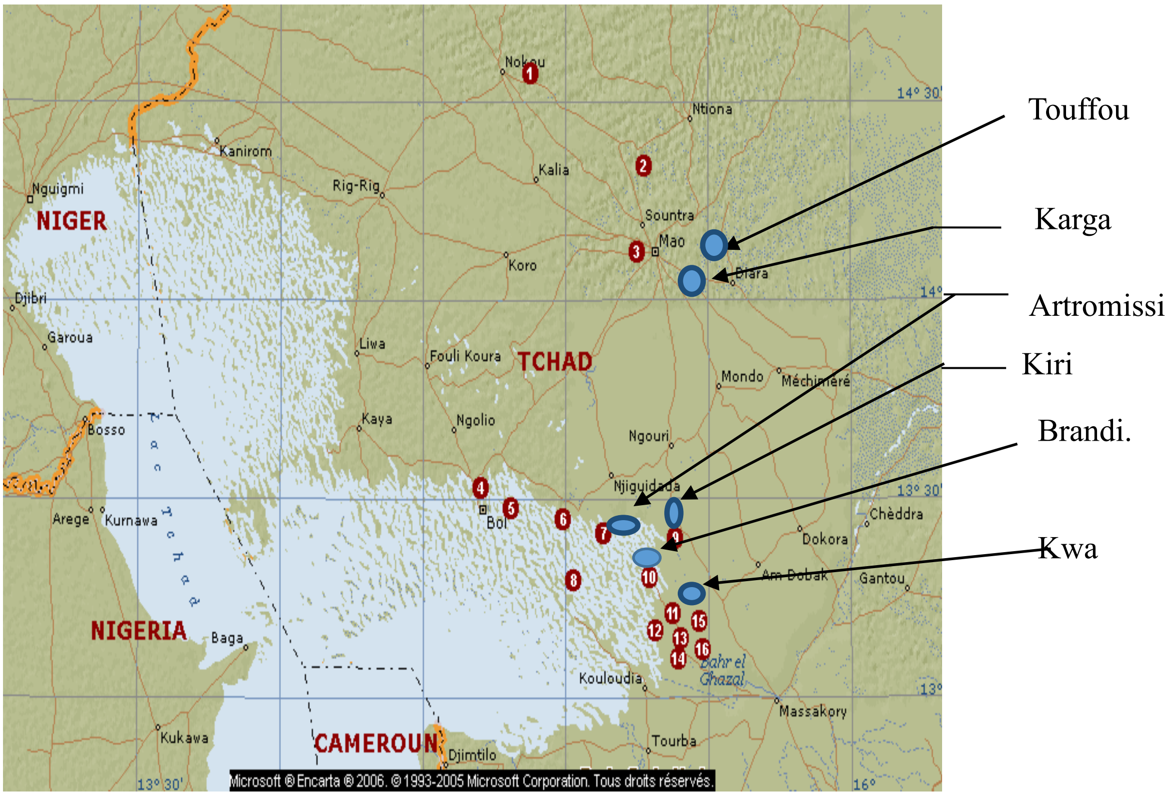

Fig. 1. Location of naturals sites of Spirulina production (Adapted from Sodelac/Tractebel, 2000).

Analytical methods

Extraction of phycocyanin from spirulina samples: Phycocyanin was isolated from Spirulina samples using maceration and sonication extraction methods. During maceration, phycocyanin was extracted from 1:25 (w/v: Spirulina powder distilled water) at 40°C for 24h. Sonication consisted of irradiating 1:25 (w/v: Spirulina powder distilled water) at 40 kHz for 40 min to optimize the isolation of C-phycocyanin (C-PC). The resulting compound from both methods was centrifuged at 10.000g for 15 min at 4°C to remove cell debris. The precipitate was discarded, while the supernate crud extract was collected. The pH of the crud extract was adjusted to 7.0 for the next steps. Purification of C-phycocyanin was performed by Ammonium sulphate precipitation as recently described Silva & Barbosa (2009). Ammonium sulphate was gradually added in 100 ml crude extracts to achieve 25% and 50% saturation with continuous stirring. The obtained solution was kept for 2 h, and centrifuged at 12000g for 30 min until the blue precipitate formed was dissolved in 0.005 M Na-phosphate buffer, pH 7.0 (Silva & Barbosa., 2009). At each extraction step, the crude phycocyanin was concentrated using the method described by Boussiba & Richmond (1979). The purity of the extracted product was calculated as indicated by Bennett & Bogorad (1973).

Dialysis and gel filtration: The crude phycocyanin was dialyzed overnight against 1000 volumes of 0.005 M Na-phosphate buffer (pH 7.0) at 40°C. Dialyzed samples were further purified by passing through a Sephadex G-25 column (12×2 cm). The column was pre-equilibrated and eluted with the same buffer. Fractions were collected at a 0.5 ml/min flow rate (Liao *et al.*, 2012), while the purity of crude phycocyanin fractions was checked using the equation:

$$\%\ \text{Crude phycocyanin} = \frac{\text{A 620nm x}(10)\ \text{x}(100)}{7.3\times(\%\ mg\ dry\ weight\ sample)}$$

where: 7.3 is the extinction coefficient of C-PC at 620 nm; 10 is the total volume of buffer (ml).

For the maximum absorption between 610 and 620 nm, C-PC generally appears in cobalt-blue (Sidler, 1998). The C-PC content in Spirulina sample was given by the equation bellow:

$$\%\ \text{C-PC-purified} = \frac{\text{A 620nm x 10 x 100}}{3.39\times(\%\ mg\ dry\ weight\ sample)}$$

where: 3.39 is the extinction coefficient of C-PC at 620 nm; 10 is the total volume of buffer (ml)

Determination of protein and amino acids contents: Total protein content in the lyophilized spirulina samples was determined by Kjeldahl method (Kjeldahl, 1883). Amino acid profile was assessed according to White *et al.,* (1986), while tryptophan was measured using HPLC technic of Anon., 2000). In short amino acids were detected by the ninhydrin reaction, while the identification was made possible using their retention time and their molecular weight. All amino acids were quantified by absorption at 570 nm, except for proline at 440 nm (White *et al.*, 1986).

Statistical analysis

All data were subjected to Analysis of Variance (ANOVA) using a Statgraphic 5.0 plus program. Means were compared between study sites through the least significant difference (LSD) test.

Table 1. Variation of non-essential amino acid contents from sampling sites (g/100g dry weight protein).

Amino acids	Karga	Sampling sites							*LSD*
		Kiri	Touffou	Kwa	CST1	Madjorio	Amerone	Artomissi	
Alanine	1.79c	1.97d	1.38b	3.13f	2.99e	0.87a	1.79c	2.99e	0.483
Arginine	2.16c	2.32d	1.51b	3.98f	3.58e	0.45a	2.20c	3.28d	0.036
Asparagine	3.16c	3.74d	2.61b	5.74e	5.56e	1.13a	5.95e	5.47e	0.293
Glutamine	5.56c	5.54c	3.73b	10.78f	9.14e	2.44a	5.92c	7.90d	0.017
Glycine	1.34c	1.45c	1.05b[f]	2.26e	2.11d	0.67a	1.37c	2.12d	0.047
Proline	1.26c	1.23c	0.93b	1.95d	1.90d	0.54a	1.27c	1.81d	0.545
Serine	1.68c	1.57c	1.22b	2.99e	2.43e	0.66a	1.75c	2.15d	0.026
Tyrosine	1.35b	1.36b	0.94a	2.55e	2.12d	0.50a	1.39b	1.94c	0.483

For each amino acid values in a line affected by the same letters are not significantly different between the sampling sites

Results and Discussion

The quality of proteins depend normally on its essential amino acid contents.

Non-essential Amino acid contents: Tyrosine content was significantly higher (p<0.001) in Spirulina from Kwa and CST1 compared to all the other sampling sites, with 2.55 and 2.12 g/100g dw respectively (Table 1). The lowest tyrosine content in Spirulina was recorded at Madjorio (0.50 g/100g dw). Tyrosine was indicated to be 1.8 g/100g dw in Spirulina samples growing on Na_2SeO_3 rich medium (Pronina *et al.*, 2002), although lowest concentration of tyrosine (0.58 g/100 dry weight) was recorded in Spirulina samples from Egypt (Babadzhanov *et al.,* 2004). Higher concentrations of tyrosine in Spirulina samples have been reported by several authors: 3 g/100g dw (Anon, 1982); 4.30 g/100 dw (Jacquet, 1974); 4.90 g/100 g dw (Clement, 1975). The variation of Tyrosine content in Spirulina could be attributed to the salinity of growing medium, which might affect synthesis of protein in Spirulina Zeng & Vonshak (1998).

Elevated serine content in Spirulina was obtained from Kwa (2.99g/100g dw), CST1 (2.43 g/100 g dw), and Artomissi (2.15 g/100 dw) samples. These contents were significantly (p<0.01) greater than those from other sites, Madjorio being the site recording the lowest serine content in (0.66 g/100 g dw). Serine contents of between 2.1 and 3 g/100 g dw were also recorded in Na_2SeO_3 rich media Pronina *et al.,* (2002). Proline content from Madjorio Spirulina samples was 0.541 g/100 g dw, significantly lower (p<0.01) than that of Kwa site (1.95 g/100 g dry weight). However, a more elevated proline content was found to be 3.06 g proline in 100 g dry weight. Some amino acids such as proline arginine were reported only in summer not in winter (Uslu *et al*., 2009). Glutamine content in different samples was significantly (p 0.01) weak at Madjorio (2.44 g/100 dw) compared to that of Kwa (10.78 g/100 g dw), value close to 9.1 g/100 g dw was also reported by Falquet and Hurni (2006). This variability should be attributed to harvest time and the photoperiodic step as affected by light intensity (Vonshak *et al*., 1996; Pandey *et al*., 2010). Glycine content in Spirulina ranged between the lowest (0.67g/100 g dw) and the highest (2.26 g/100 g dw) values obtained respectively from Madjorio and Kwa respectively. Values of up to 3.2 g/100g dw was previously reported (Jourdan, 2006). Spirulina samples harvested from Kwa, CST1, Amerone and Artomissi had significantly (p<0.01) higher contents in asparagine compared to that of other sites, as 5.74, 5.56, 5.96, 5.47 g/100g dw protein respectively. Sample from Madjorio contained the lowest asparagine content (1.13 g/100 g dw protein). Our results are different from those of other researchers who have obtained 6.1g of asparagine in 100 g dry weight protein (Anon., 1982); Jourdan, 2006). The arginine contents in Spirulina ranged between 3.98 g/100 g dw at Kwa samples (significantly higher than that other sites (p<0.01) to 0.45 g/100 g dw in Madjorio samples. Although more elevated values such as 4.3 g/100 g dw were also reported (Marrez *et al*., 2014), lower values were also recorded in winter eg.1.54 g/100 g dw (Uslu *et al.,* 2009). Alanine content was more abundant in Spirulina samples from CST1 and Artomissi sites, with 2.99 g/100 g dw protein. The smallest alanine content was again encountered in Madjorio Spirulina samples (0.82 g/100g dw). Values ranging from between 1.77 and 2.18 g/100 g dw were obtained by Uslu *et al*., (2009). Cysteine and Tryptophan were not found in any of the Spirulina samples collected at different sites. Whereas 0.09 g cysteine /100g dw or 0.15 g tryptophan /100 g dw were found in Spirulina (Borowitska, 1988), tryptophan content of 0.08 g/100g dw was revealed in other samples (Babadzhanov *et al.,* 2004), but not Cysteine. Tryptophan was the amino acid found in lowest content between 0.06 and 0.082 g/100 g dw protein (Volkmann *et al*., 2008). Amino acids poorly present in Spirulina have been claimed as the sulphur amino acids (Girardin, 2005).

Essential amino acid content in spirulina: The eight amino acids present in proteins are also called essentials amino acids that cannot be synthetized by by *S. platensis,* and must be produced as a supplement (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, histidine, valine). Figure 2 shows the composition of essential amino acid found in Spirulina of our different sampling sites. Phenylalanine content varied significantly (p<0.01) from one production site to another, with 2.43 g of phenylalanine in 100 g dry weight of Spirulina originated from Kwa, and only 0.56g/100 g dry weight from Madjorio site. Our results are lower than 5.8 g of phenylalanine found in 100 g dry weight spirulina grown on artificial medium Babadzanov *et al*., (2004).

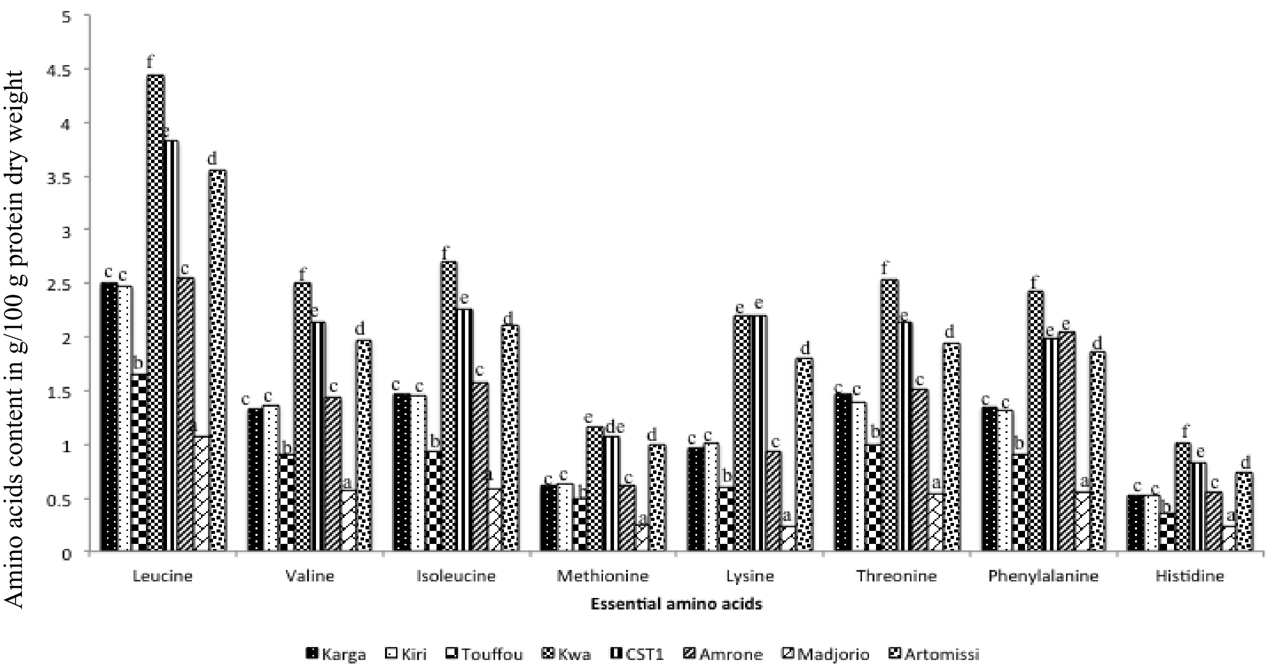

Fig. 2. Differences in essential amino acid contents in Spirulina sampled from study sites For each amino acid bars affected by the same letters are not significantly different between the sampling sites.

Analysis of different samples indicated that the highest content of threonine (2.54g/100) in Spirulina samples was obtained at Kwa and the lowest (0.54g/100) at Madjorio, with respectively 2.54 and 0.54 g/100 g dry weight. Threonine contents between 1.10-2.00 g/100 g dry weight were reported in Spirulina in winter and summer, compared to 0.94-1.36 g/100 g dry weight pointed out by Uslu *et al.,* (2009). Kwa and CST1 samples significantly ($p = 0.01$) indicated high content in lysine (2.20 g/100 g dry weight), whereas the lowest accouted for Madjorio site (0.23 g/100 dry weight). Many authors agree on the fact that lysine is poorly represented in Spirulina (Borowitska, 1988; Quillet, 1975), while others consider its content in acceptable limit (Clement *et al.*, 1975). Our results are very low compared to those of other authors, Falquet & Hurni (2006) obtained 3.2 g/100 g dry weight of Spirulina.

Methionine was the lowest essential amino acid in proportion in all sites. It was very low in Madjorio samples (0.25 g/100 dry weight, but high in Kwa samples (1.17 g/100g dry weight). Other authors have obtained higher values with respectively 0.8 g (Babadzhanov *et al.,* 2004), and between 0.16-0.52 g (Marrez *et al.,* 2014) of methionine in 100 g dry weight of Spirulina. Leucine was the only essential amino acid found to be relatively stable in all the Spirulina samples. The lowest content was 1.07 mg/100 g dry weight found in Madjorio sample, while the highest content was that of Kwa samples (4.43 g/100g dry weight). Our results differ from those of Uslu *et al.,* (2009), who reported values of between 1.86-3.19 g Leucine /100g dry weight of Spirulina protein. Up to 5.4 g/100g dry weight of leucine in Spirulina was recorded by Jourdan (2006). Whereas Leucine (6.17 g/100 g dw protein), and valine (4.21 g/100 g dw protein) were found to be the major essential amino acids, tryptoplan (0.85 g/100 g dw protein) was revealed as the lowest amino acid in content encountered in Spirulina (Bashir *et al.,* 2016). Histidine was present in Spirulina at variable contents ranging from 0.25g/100g dry weight (sample from Madjorio) to 1.01g/100 g dry weight (sample from Kwa). The recent report by Marrez *et al.,* (2014) has revealed Histidine content of between 0.74 g/100 and 1.35 g/100 g dry weight of Spirulina protein.

Valine found in Spirulina has a content varying between 2.50g/100 g dry weight and 0.57g/100 g dry weight, respectively from Kwa and for Madjorio samples. Lower (0.13 g/100 g dry weight), or higher (4.0 g/100g dry weight) values were respectively reported Babadzhanov *et al*., (2004). As far as isoleucine content in Spirulina was concerned Madjorio site showed the smallest values, whereas the highest accounted for Spirulina from Kwa site (2.70 g/100 g dry weight). These results are not enough compared to 3.5 g of isoleucine obtained in 100g dry weight of Spirulina Falquet & Hurni (2006). Glutamic acid (8.47 g/100 g dw protein) was pointed out as the major non-essential amino acids, unlike cysteine (0.72 g/100 g dw protein) that was the lowest in content (Bashir *et al*., 2016). Cereals are generally low in limiting amino acids such as lysine and tryptophan, whereas these amino acids appear in relatively high amount in spirulina. This attribute has enabled Spirulina to be used as suplement in cereal based diets (Anon., 2006).

The synthesis of essential amino acids in Spirulina may largely depend on the composition of nutrients in growing media. Only two essential amino acids (lysine, tryptophan) were reported to be present in Spirulina, with concentration below the FAO's minimum requirements when Spirulina was produced in desalinator waste water (Volkmann *et al*., 2008). The presence of all amino acids in all our Spirulina samples is an indication that the sampling sites offer good growth conditions to Spirulina. Tryptophan content in Spirulina has been reported to be usually low, with contents ranking from 0.139-0.144 g/100 g dw protein (Campanella *et al*., 1999).

Total proteins content of Spirulina from different sites: The total protein content of Spirulina comprised between 39.55 and 64.26g/100g dw from Madjorio and Kwa sites respectively (Fig. 3). A total protein content 52.95 g /100g was recorded in Spirulina, harvested from the fields, whereas fields containing ammonium nitrate had total protein content ranging from 44. 07 to 52.62g/100 g dw (Fedekar *et al.*, 2012). According to the same authors, fields enriched with urea have resulted in decreased protein content of 37.79 g/100g dw in Spirulina, similar to what was observed at Madjorio due to the high sodium content in this sites. The protein content of between 60-61 g/100g dw in Spirulina was reported by Pronina *et al.,* (2002) which falls within the range 39.55- 64.26 g/100g dw obtained in this study. A decrease in proteins content from 44.1-36.1g/100g dw was reported when NaCl concentrations in the growing medium was between 0.50M and 0.75M (Vonshak *et al.,* 1996).

Our results lined with those of Ngakou *et al.,* (2012), who noticed a total protein content from 58.61 g/100 dw in Spirulina harvested from Artomissi site. A traditional spirulina was reported to contain 60.6-61.4% protein (Anon., 2008), compared to 63.3-69.40% reported by Anon., (2009). Results obtained from this study are similar 55-65 mg/100g dw, indicating that Spirulina is a plant known for its richness in vegetable proteins Banks (2007), but our values were lower than 69.2 g/100g dw obtained by Mbaiguinam *et al.,* (2006), although closer to 62.5% to Indian Spirulina Venkataraman *et al*., (1997), but not far from 66.6% found in samples of Spirulina grown in USA (Grinstead *et al*., 2000). This protein content variability among samples from various origins has been attributed to the harvesting period, as well as the daily brightness (Van & Shilo, 1986). This could also be attributed to high temperature occuring at the site. An increased temperature from 35-42°C was able to stimulate changes in the macromolecular composition of Spirulina, with high temperature reducing protein content from 64 to 48% (Koru & Cirik, 2002).

Phycocyanin Contents of Spirulina harvested from different sites: Spirulina contains phycocyanin, β-carotene and xanthophyll pigments, α-tocopherol and phenolic compounds, which are responsible for the antioxidant activities of these microalgae, as shown by several authors for in vitro and in vivo experiments (Patel *et al*., 2006). Phycocyanin was reported to be a potent free radical scavenger that inhibits microsomal lipid peroxidation (Pinero *et al*., 2001). Phycocyanin contents from Spirulina samples harvested at CST1 and kwa sites were the highest 15.80 mg/100 dw and 15.72 mg/100g dw respectively (Fig. 4). The lowest concentration of phycocyanin in Spirulina was 2.81mg/100g dw recorded at Amerone.

The pure phycocyanin contents obtained were 34.02 and 33.81mg/100 g dry weight for the Kwa and CST1 site respectively. The lowest content was encountered at Amerone site (6.06 mg/100g dry weight of Spirulina) and Madjorio (6.66 mg/100 dry weight of Spirulina). A pure phycocyanin content of between 10-46.43 mg/100 g dw was reported by Ngakou *et al*., (2012). Phycocyanin contents between 15-20 mg/ 100 g dw and 15-22 mg/100g dw were also found in Spirulina by Pierlovisi (2007) and Henrickson (1997), respectively with the most elevated content of between 24 and 33.8 mg/100g dw was reported by Ratana *et al*., 2007).

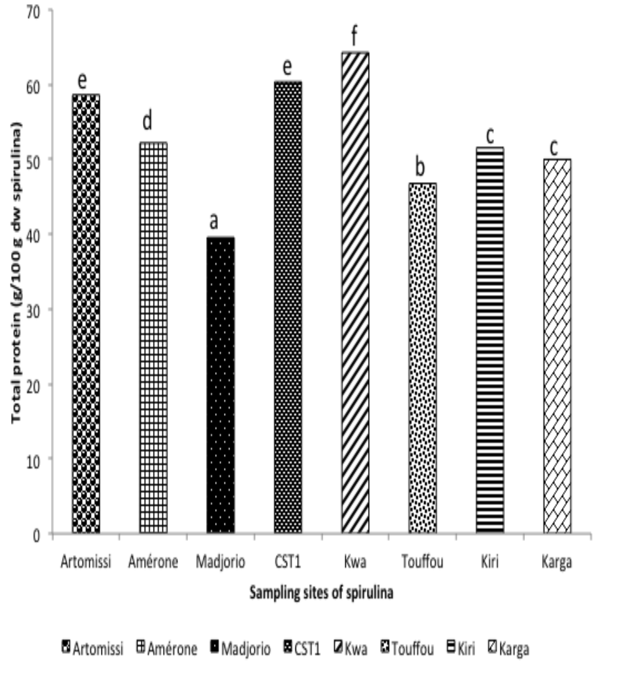

Fig. 3. Changes of proteins contents in Spirulina as affected by sampling sites Bars affected by the same letters are not significantly different between the sampling sites.

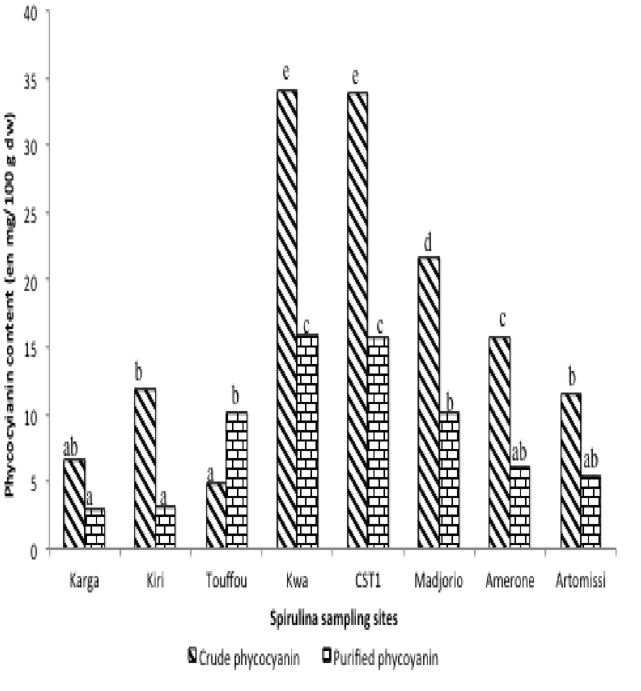

Fig. 4. Variation of phycocyanin content in Spirulina as affected by sampling sites for each phycocyanin type, bars affected by the same letters are not significantly different between the sampling sites.

Conclusion

At the end of this study, Spirulina sampled from different sites did have nutritional values in terms of their richness in essential and non-essential amino acids, proteins and phycocyanin. Spirulina harvested from Kwa, CST1 and Artomissi sites were nutritionally more valuable than the ones from other sites. Cysteine and tryptophan were the only amino acids absent in all Spirulina samples. Madjorio was the poorest of all the sites in amino acids, protein and phycocyanin because of the richness of the site in sodium. The results of this study have clearly indicated that the biochemical composition of Spirulina is influenced by the sampling sites, and within the sampling sites by the natural or atificial growth conditions.

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