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Storage influence on beta-carotene and alpha-tocopherol contents of solar-dried *Spirulina platensis* (Spirulina)

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Spirulina is called the ideal food for mankind and the World Health Organization considered it "super food" and the best food for the future because of its high nutritional value. The present study aimed to assess the storage influence on the levels of β-carotene and α-tocopherol in solar-dried spirulina. It was an analytical study to determine the levels of β-carotene and α-tocopherol in solar-dried spirulina using high performance liquid chromatography (HPLC). The mean contents of β-carotene and α-tocopherol of spirulina were 57.38 ± 9.98 and 1.72 ± 0.51 mg/100 g, respectively. After six months of storage, losses in β-carotene and in α-tocopherol were 24 and 28.49%, respectively. Drying and conditioning of spirulina remain the only means for broad commercial distribution. The nutritional losses that occurred during the storage of the spirulina suggest early consumption after harvesting and some special measures for conditioning and storage.

Key words: Spirulina, α-tocopherol, β-carotene, farming, nutritional value.

INTRODUCTION

Spirulina (*Spirulina platensis*, family of *Oscillatoriaceae*) is one of the blue-green algae rich in protein (62.84%) and contains a high proportion of essential amino acids (38.46% of the protein) and is rich in vitamins especially vitamin B complex such as vitamin B12 (175 µg / 10 g) and folic acid (9.92 mg / 100 g), which helps the growth and nutrition of the child brain. It is also rich in calcium and iron (922.28 and 273.2 mg / 100 g, respectively) to protect against osteoporosis and blood diseases, as well as a high percentage of natural fibers (Sharoba, 2014). So, the spirulina is useful and necessary for the growth of infants and very suitable for children, especially in the growth phase, the elderly and the visually impaired. It also helps a lot in cases of general weakness, anemia and chronic constipation. Spirulina contains selenium (0.0393 mg/100 g) and many of the phyto-pigments such

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as chlorophyll and phycocyanin (1.56 and 14.647%), that are known to be powerful antioxidants (Sharoba, 2014). It has been experimentally proven, *in vivo* and *in vitro* that spirulina is effective to treat certain allergies, anemia, cancer, hepatonephrotoxicity, viral and cardiovascular diseases, hyperglycemia, hyperlipidemia, immuno-deficiency, and inflammatory processes, among others (Chamorro et al., 2002; Romay et al., 2003, Selmi et al., 2011, Abdel-Daim et al., 2013, Ouédraogo et al., 2013, Ibrahim et al., 2015, Abdel-Daim et al., 2015). Thus, spirulina is considered generally recognized as safe (GRAS), without toxicological effects, and it is approved by the FDA (USA) and ANVISA (Navacchi et al., 2012). The U.S. Food and Drug Administration (1981) has not questioned the basis for the Generally Recognized as Safe designation to spirulina under the conditions of its intended use, thus restricting it as a food additive in amounts that range from 0.5 to 3.0 grams per serving. Formulators use spirulina in specialty food bars, powdered nutritional drinks, popcorn, beverages, fruit and fruit juices, frozen desserts and condiments (Sharoba, 2014).

During the last decade, farming and consumption of this alga is booming in all developing countries (Charpy et al., 2008). Its consumption is indicated as one of the solutions in the fight against multi-nutrient deficiency in these countries (Simporé et al., 2007; Charpy et al., 2008; Teas and Irhimeh, 2012). This dietary supplement offers remarkable health benefits to undernourished children. It is rich in beta-carotene that can overcome eye problems caused by vitamin A deficiency; it provides the daily dietary requirement of beta-carotene which can help prevent blindness and eye diseases (Seshadri, 1993). The protein and B-vitamin complex makes 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 and GLA that helps to regulate the entire hormone system (Ramesh et al., 2013). Spirulina contains also α-tocopherol (Falquet and Hurni, 2006) which is known in most cases for its antioxidative properties and its involvement in the regulation of oxidative stress (McLaren and Frigg, 2001; Traber and Atkinson, 2007; Abbes et al., 2013; Rajendran et al., 2013). Consequently, spirulina constitutes an interesting source of β-carotene and of α-tocopherol which must both be controlled during the production and processing.

In Burkina Faso, multi-nutrient deficiency is a public health problem. According to the demographic and health survey in 2010, the manifestations of malnutrition among children of less than 5 years are stunting (35%), under-weight (16%), and high rate of anemia (88%) (INSD, 2010). One of the solutions undertaken in the fight against nutritional deficiencies is the introduction of spirulina farming and the popularization of its consumption in small doses as a dietary supplement (Sawadogo et al., 2004). The main objective of farms involved in the spirulina production is to contribute in improving the community's health through the use of spirulina (Charpy et al., 2008). Burkina Faso government approves this goal by funding entirely the Koudougou integrated spirulina production project "Nayalgué" (Sawadogo et al., 2004; Charpy et al., 2008).

Fresh spirulina remains less accessible to the population because of short shelf life. The drying of fresh biomass and its storage remain the only sure means for commercialization or humanitarian distribution. The drying enables a stabilization of the hydrated spirulina through reduction of both water content and activity. However, chemical modifications are likely to occur in dry biomass during the drying and storage. The oxidative components such as α-tocopherol and β-carotene are sensitive to atmospheric oxygen, to light and to variations of temperatures (Seshadri et al., 1991; McLaren and Frigg, 2001; Rastrelli et al., 2002; Cuvelier et al., 2003; Ferreira et al., 2008). These processes negatively influence β-carotene and α-tocopherol content in spirulina. Moreover, little is known about storage impact on nutritional value of spirulina commercialized and produced in Burkina Faso. The purpose of the present study was to assess the influence of storage on β-carotene and α-tocopherol contents in solar-dried spirulina.

MATERIALS AND METHODS

Sampling

Samples of spirulina were collected from the experimental farm of the Research Centre in Food, Nutritional and Biological Sciences (CRSBAN), located at the University of Ouagadougou, Burkina Faso. Spirulina was produced in two rectangular basins with a depth of 50 cm, 12.5 m^2 of surface and a total volume of 6250 L each. This farm, approved by West African *Economic and* Monetary Union (*WAEMU)* was undertaken in order to develop biotechnology products used for enriching dietary protein and other micronutrients such as vitamins for malnutrition control and for People Living with HIV (PLHIV). Spirulina (*Spirulina platensis*, var. "Lonar") was harvested after a growing period of two weeks. All parameters of production, that is temperature, salinity, water level, biomass density were monitored daily. Briefly, harvesting was performed on the basis of biomass density determined using a Secchi disk. Secchi disk value is a spirulina growth indicator (Jourdan, 2010). When this value is low, spirulina growth and biomass density increase in the basin. Harvesting was done based on Secchi disk 3 cm minimal value by pouring the algal suspension on cotton cloth filter supported by the basins. Harvesting time was done early in the morning at 5 o'clock. Fresh spirulina were harvested at this moment in order to reduce sunlight impact in the biomass quality. The filtrate was collected in the basin and the harvested slurry was manually wrung, and pressed in a screw press. A total of twenty (20) samples of fresh spirulina were harvested, 10 samples per basin.

Drying

The biomass obtained after pressing was extruded using SIKA pistol. This device consisted of a cylinder, which was filled with squeezed spirulina, and a piston rod that was pushed to force spirulina out at the other end through a disk with holes of 2 mm diameter. This pathway gave rise to "spaghettis" that were spread

in strips on a rack for sun drying. Spirulina strings were not touched or overlapped, to allow free passage of air and accelerate the removal of the residual moisture. Sun drying was carried out for 5 h using a dryer box type “coquillage” (Ferradji et al., 2008). After drying spirulina spaghettis, they became hard and brittle, easier for grinding. The end of drying was determined by the cracking characteristic of the spirulina “spaghettis” when crushed in the hand. After drying, all samples were removed from dryer and placed in an aluminum plate. Dried biomass was immediately weighed and ground.

Storage

The powder conditioning was done in a room with closed windows at ambient temperature (30°C). The powder was manually conditioned in aluminous plastic bags (10 g/sachet). Briefly, the powder was inserted into the bags using a spoon, weighed and manually sealed. The powder samples were immediately transported in a cooler bag to the chemistry and toxicology laboratory of the University of Ouagadougou where chemical analyses of nutrients were undertaken. The 20 powder samples were analyzed immediately upon arrival (zero time) in order to determine the α-tocopherol and β-carotene contents. Collected samples were stored at the ambient temperature of 30°C and sheltered from air and light in a dryer box until the second analysis performed after six months of storage (Month 6). The analyses after 6 month's storage were performed on the same samples numbered from 1 to 20. This approach aimed to verify the impact of medium-term storage on the β-carotene and α-tocopherol stability. The packing and the conditions of sample storage in the laboratory aimed to reduce nutritive losses.

Determination of β -carotene and α-tocopherol

β-carotene and α-tocopherol contents of the samples were determined using the method of Somé et al. (2004). The analyses were performed using the high performance liquid chromatography (HPLC) system which consists of a JASCO PU- 980 pump (Tokyo, Japan) equipped with a 20 µL loop injection, a chromatographic column Supelcosil LC-18 (Bellefonte, USA) of 25 cm length, 4.6 mm in diameter and a particle size equal to 5 µm. The mobile phase used is a ternary mixture consisting of methyl alcohol (95% v/v), acetonitrile (3% v/v), and water (2% v/v). The mobile phase flow rate was set at 2 ml per minute. The α-tocopherol and β-carotene detection were carried out at 290 nm and 450 nm, respectively with a UV detector (JASCO 975, Tokyo, Japan). The system was coupled to a computer system and data processing software (Galaxy Work Station). The measurement of the optical density (O.D.) of standard solutions was done with a UV-visible spectrophotometer A-160 Type CECIL (UK).

Preparation of standards solutions for calibration

Five milligrams (5 mg) of α-tocopherol and β-carotene standards were dissolved in 3 mL of hexane. Some dilutions were performed (1/10, 1/100 and 1/1000) and the optical density (O.D.) measured at 290 and 450 nm. The solutions with optic density between 0.1 and 0.9 were kept. The concentrations were then calculated according to the following formula:

$$C = \frac{0.D.}{\varepsilon} \times 10^{-9}\ \mu g/mL$$

Where *O.D* is the optic density and ε is the molar extinction coefficient.

Standards solutions (30 pmol/20 µL) were prepared and evaporated under nitrogen. The residue was re-suspended in 1 mL of acetonitrile before injecting into the chromatograph to identify the standards peak heights.

Extraction of vitamins from samples of solar-dried spirulina

To extract vitamins from spirulina, 10 mg of dried spirulina powder were mixed with 1 mL of ethyl alcohol, 4 mL of hexane and 1 mL of sodium chloride 3 M solution and kept at 4°C for 24 h. The mixture was centrifuged at 3000 rpm for 10 min to separate the hexanic phase containing vitamins and the aqueous phase. Hexanic extract (4 mL) was further extracted with 1 mL of diméthylformamide, vortexed and centrifuged at 3000 rpm for 10 min. A portion of the extract (1 ml) was transferred to a 10 ml glass tube, dried under nitrogen, and re-suspended with 1ml of acetonitrile for HPLC analysis. Twenty microliters (20 µL) were injected into the HPLC. Two injections were performed and the average peak area was used for the calculation of vitamins content (Somé et al., 2004; Mills et al., 2009).

Vitamins contents determination in solar-dried spirulina

β-carotene and α-tocopherol peaks were identified and measured on the chromatogram on the basis of the retention times for the specific standard located around twelve (12) min and five (5) min respectively. For each sample of spirulina, two (2) tests were performed (Figure 1). For each test, the injections were duplicated and the average area of the two (2) resulting injections was subsequently considered for calculation (Figure 2). The data were analyzed and processed using Microsoft Office Excel 2010. A calibration mixture including an internal standard with defined concentrations was injected; a calibration factor was then calculated for each peak as follows:

$$Fi = \frac{AUCe \times Ns}{Ne \times AUCs}$$

Where, *Fi* is the calibration factor; *AUC*e is the Area under curve for the sample; *AUCs* is the Area under curve for the standard; *Ns* is the number of pmol injected for vitamins standards and *Ne* is the number of pmol injected for samples in the following formula:

$$Ne = \frac{AUCe \times Ns}{Fi \times ASCs}$$

The calculation of vitamins contents were done with the average of two samples weighs tests according to the following formula:

$$T = \frac{Ne \times F \times Pm \times AUCe \times 10^{-9}}{AUCs \times Pe}$$

with *T* the content of vitamins (mg/100 g), *Pm* is the molecular weight for vitamins standards in g, *P*e is the sample weight test in g, 10^{-9} is the factor of conversion to pass from pmol to mg and *F* is the dilution factor of the sample.

Data and statistical analyses

Data were recorded and validated using Epi Data software (http://www.epidata.dk/), then analyzed using SPSS version 17.0 (International Business Machines Corporation, Armonk, New York, USA). Word Software and Excel 2010 were used for the treatment

Table 1. Means contents of vitamins at zero time (M0) and after 6 months of storage (M6).

N° of samples	β-carotene (mg / 100 g) (n=2)		α-tocopherol (mg / 100 g) (n=2)	
	Zero time (M0)	M6	Zero time (M0)	M6
1	44.13 ± 0.73	32.85 ± 1.51	1.73 ± 0.17	1.25 ± 0.04
2	65.96 ± 1.20	40.42 ± 1.51	2.11 ± 0.15	1.57 ± 0.03
3	46.32 ± 1.55	37.87 ± 0.89	1.47 ± 0.07	1.14 ± 0.02
4	47.26 ± 2.33	33.04 ± 2.11	1.68 ± 0.04	1.38 ± 0.06
5	72.80 ± 1.25	49.39 ± 0.35	2.91 ± 0.01	2.15 ± 0.01
6	48.42 ± 1.91	41.36 ± 0.96	1.71 ± 0.05	1.06 ± 0.03
7	54.99 ± 1.25	51.01 ± 1.27	1.46 ± 0.06	0.98 ± 0.03
8	52.63 ± 0.54	38.19 ± 0.43	1.31 ± 0.08	0.88 ± 0.01
9	74.32 ± 1.41	61.92 ± 0.56	2.34 ± 0.01	1.53 ± 0.02
10	65.85 ± 1.40	53.05 ± 0.96	2.46 ± 0.03	1.72 ± 0.02
11	42.72 ± 0.54	34.65 ± 0.61	1.20 ± 0.05	0.82 ± 0.01
12	50.55 ± 0.94	42.76 ± 0.92	1.32 ± 0.08	1.12 ± 0.01
13	66.85 ± 2.00	48.53 ± 2.00	1.27 ± 0.05	0.93 ± 0.02
14	62.48 ± 1.14	45.12 ± 0.57	2.06 ± 0.04	1.69 ± 0.06
15	55.40 ± 1.24	34.55 ± 0.44	1.29 ± 0.03	0.85 ± 0.02
16	57.66 ± 0.81	47.12 ± 1.16	1.21 ± 0.05	0.78 ± 0.03
17	45.91 ± 0.77	38.45 ± 0.29	1.08± 0.05	1.08 ± 0.03
18	60.05 ± 2.37	43.87 ± 0.68	1.69 ± 0.03	1.28 ± 0.04
19	63.49 ± 1.57	49.93 ± 0.68	1.67 ± 0.01	1.13 ±0.02
20	69.96 ± 2.16	52.57 ± 0.84	2.42 ± 0.01	1.18 ± 0.01
Means	57.38 ± 9.98	43.83 ± 7.83	1.72 ± 0.51	1.23 ± 0.36

Values are means for two independent experiments.

of the text as well as the pictures and graphs respectively. Content of β-carotene and α-tocopherol were expressed as mean of two replicates ± standard error (SE). The paired t-test was used to compare the concentrations of α-tocopherol and β–carotene during the storage of the samples. The significance level was set at $p<0.05$.

RESULTS

The initial vitamins content of *S. plantesis*

The initial β-carotene content of *S. platensis,* analyzed at zero time (M0), varied from 42.72 ± 0.54 mg/100 g to 74.32±1.41 mg/100 g and the mean was 57.38 ± 9.98 mg/100 g (Table 1 and Figure 3). The α-tocopherol content at zero time (M0) ranged from 1.08 ± 0.05 mg/100 g to 2.91 ± 0.01 mg/ 100 g with the mean of 1.72±0.51 mg/100 g.

Vitamins contents in the solar-dried spirulina at Month 6 storage

After six months of storage, β-carotene content ranged from 32.85 ± 1.51 to 61.92±0.56 mg/100 g and the mean was 43.83 ± 7.83 mg/100 g (Table 1 and Figure 3). With respect to α-tocopherol content, after six months of storage it varied from 0.78±0.03 to 2.15 ± 0.01 mg/100 g and the mean was 1.23 ± 0.36 mg/100 g (Table 1 and Figure 3). The results show that the β-carotene and α-tocopherol contents had decreased after 6 months of storage (Figures 4 and 5). The β-carotene and α-tocopherol losses were 24 and 28.49%, respectively (Table 2). As shown in Table 2, the β-carotene and α-tocopherol losses after 6 months of storage were statistically significant ($p <0.0001$).

DISCUSSION

Based on its β-carotene content, 100 g of the spirulina provides 9564 µg retinol equivalent (RE). The daily human requirement is around 450-500 µg RE (Somé et al., 2014), and the daily 10 g spirulina regimen recommended for adults (Ouédraogo et al., 2013) can cover the daily requirement necessary for efficient biotransformation from carotene to retinol.

At zero time (month 0), the variation of β-carotene and α-tocopherol contents between the different samples was revealed in this study. The variation of the β-carotene and α-tocopherol contents observed could be explained by a non-uniform drying of the fresh biomass of spirulina. The

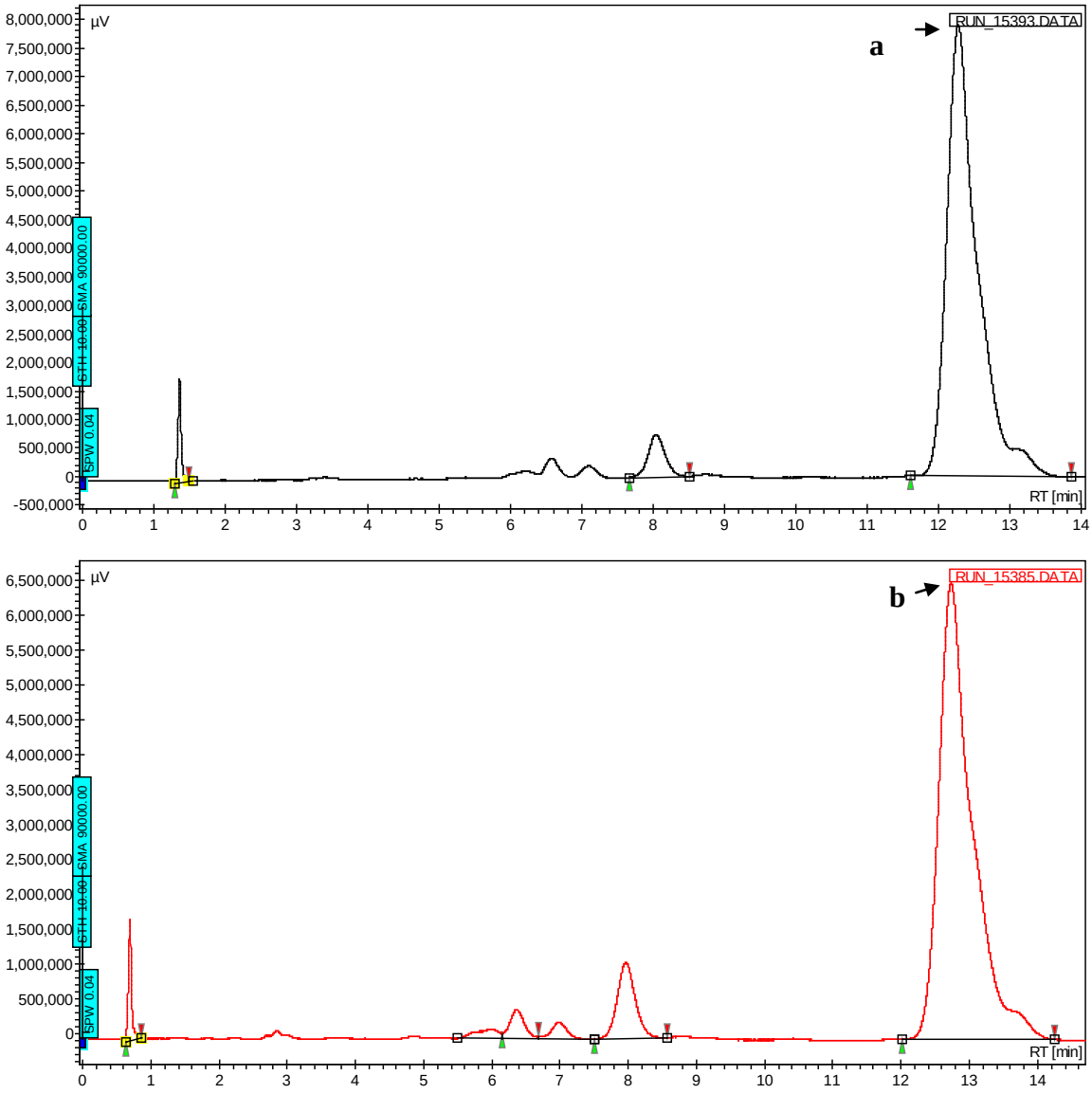

Figure 1. Chromatograms of the analysis of the twenty samples of *Spirulina* [**a**= β-carotene peak at zero time (Month 0); **b**= β-carotene peak at month 6; the retention time is around 12 min].

technique used was direct sun drying. In this regard, fresh biomass was extruded in cylinders with a die of 2 millimeters in diameter (spirulina “spaghetti”), and then spread over a screen contained in a dryer box exposed to the sun light. The temperature of air entering the drying box, which proves to be a preponderant parameter to guarantee the nutritional value of the spirulina (Beccera et al., 2005), was not controlled. Samples dried using this technique contains contents of non-homogeneous residual water which are likely to have an influence on the nutritive content (Lingani-Sawadogo et al., 2005).

The β-carotene contents obtained from this study were lower than the values reported by Careri et al. (2001), ranging from 70 to 200 mg/100 g of spirulina. The difference could be due to the drying techniques. In the solar drying used in our study, whole spirulina “spaghettis” were dried, leading to a longer drying time. With this method the inside of cells were not subjected to direct contact with hot air. These authors used drying by pulverisation where the juice of crushed spirulina was dried. In that respect, filaments were reduced beforehand down to gruel to break their membrane before being subjected to a current of gas of combustion with very high temperature for a very short time (Beccera et al., 2005; Jourdan, 2010). Our samples would probably contain more residual water than those of these authors. Moreover, the exposure of spirulina to natural conditions of temperature, relative humidity and UV light seems to

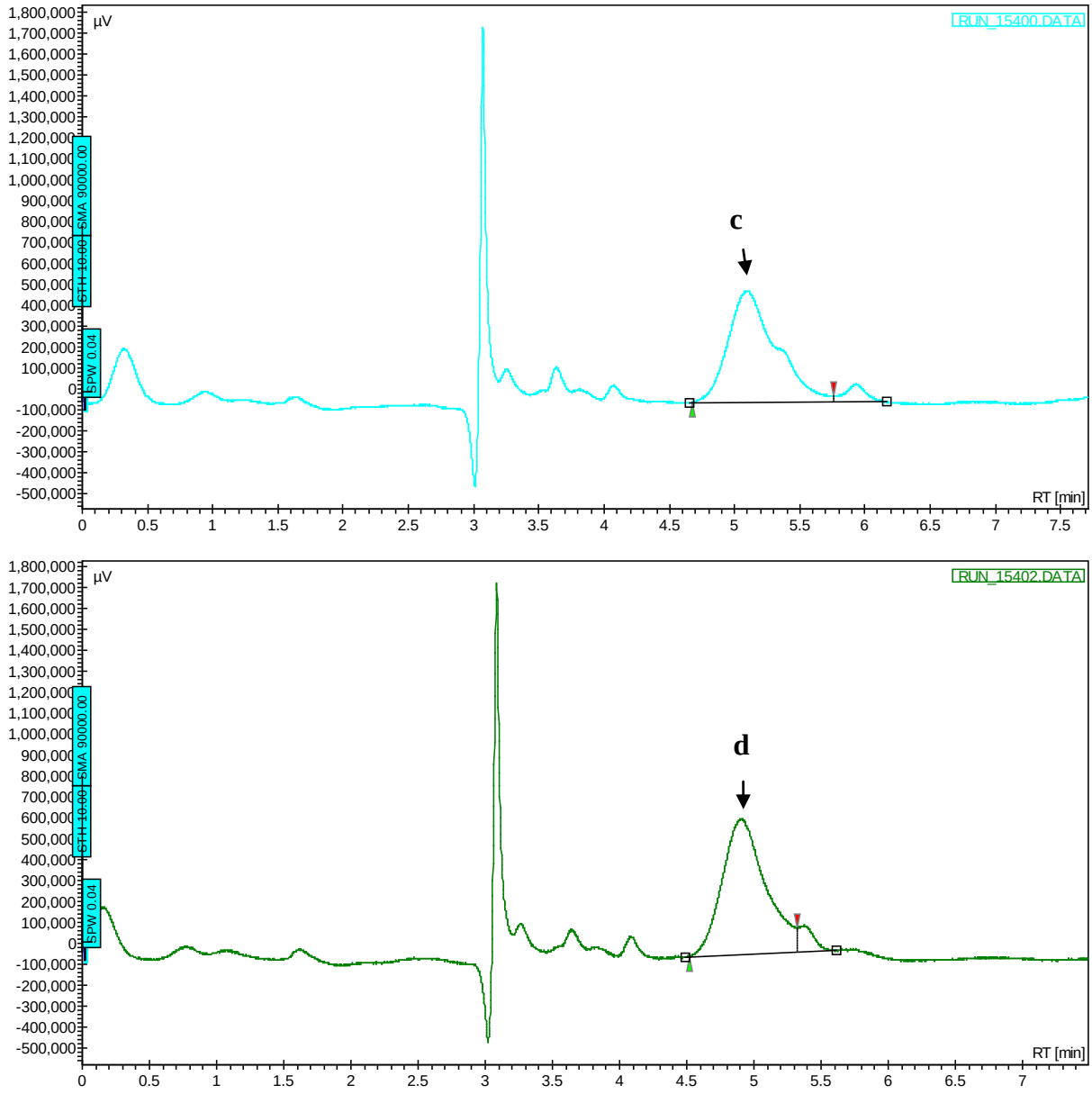

Figure 2. Chromatograms of the analysis of the twenty samples of *Spirulina* [**c**= α-tocopherol peak at zero time (month 0); **d**= α-tocopherol peak at month 6; the retention time is around 5 min].

degrade β-carotene (McLaren and Frigg, 2001; Jourdan, 2010).

The mean content of α-tocopherol in our study was close to that of Gomez-Coronado et al. (2004) who reported a value of 1.3 mg/100 g of α-tocopherol in solar-dried spirulina sold in the market (Gomez-Coronado et al., 2004). These results suggest that the solar-dried spirulina is not a suitable source of α-tocopherol for consumers whose daily needs are more than 4 mg of α-tocopherol (Chavan, 2005).

In this study, losses of β-carotene and α-tocopherol occurred during storage. After six months of storage at ambient temperature, losses in β-carotene and in α-tocopherol were 24 and 28.49%, respectively. The decrease of β-carotene and α-tocopherol contents could be due to a chemical deterioration of β-carotene and α-tocopherol through the duration (5 h of drying) and the conditions of storage. Deterioration of β-carotene and α-tocopherol during the storage has already made the object of numerous previous research work (Seshadri et al., 1991; Rastrelli et al., 2002; Ferreira et al., 2008). Moreover, it has been demonstrated that high losses of provitamin A occur during storage in ambient conditions. After four months of storage at ambient temperature, an

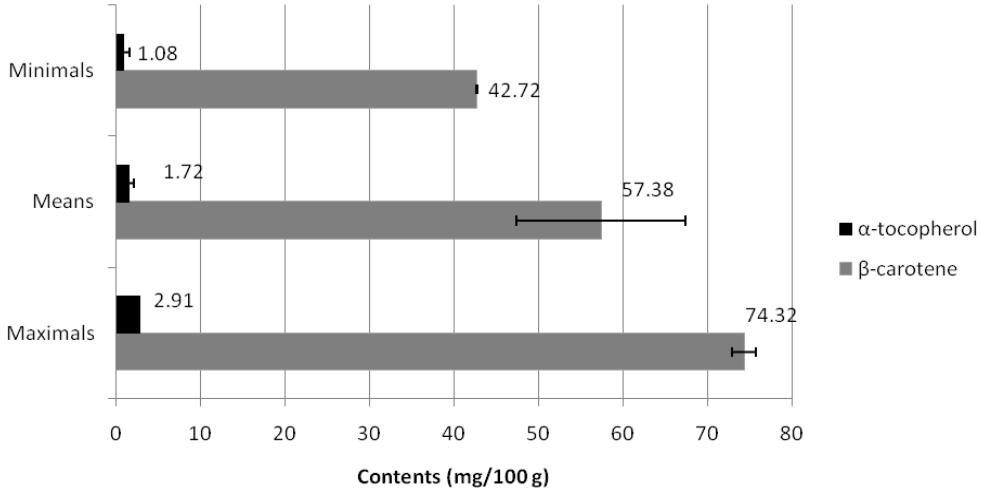

Figure 3. Variation of initial vitamins contents (mg/100 g) of *Spirulina plantensis*.

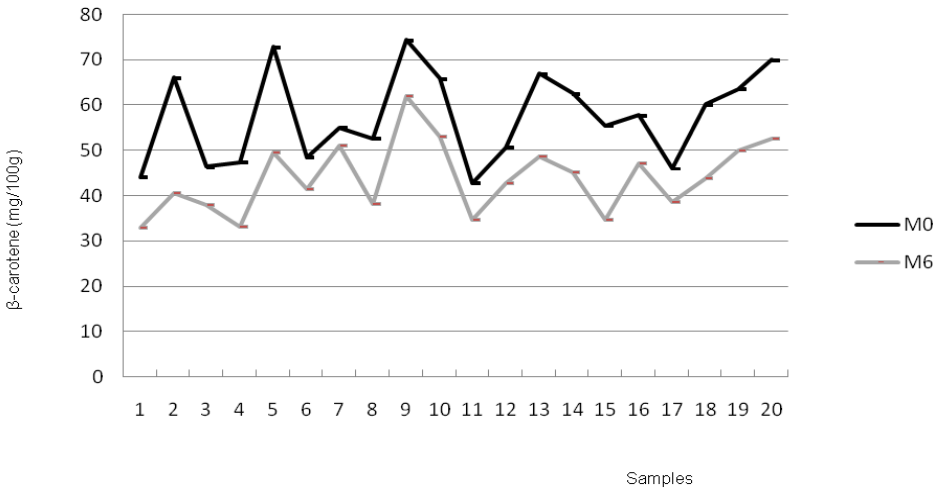

Figure 4. Variation of β-carotene contents (mg/100g) after 6 months of storage.

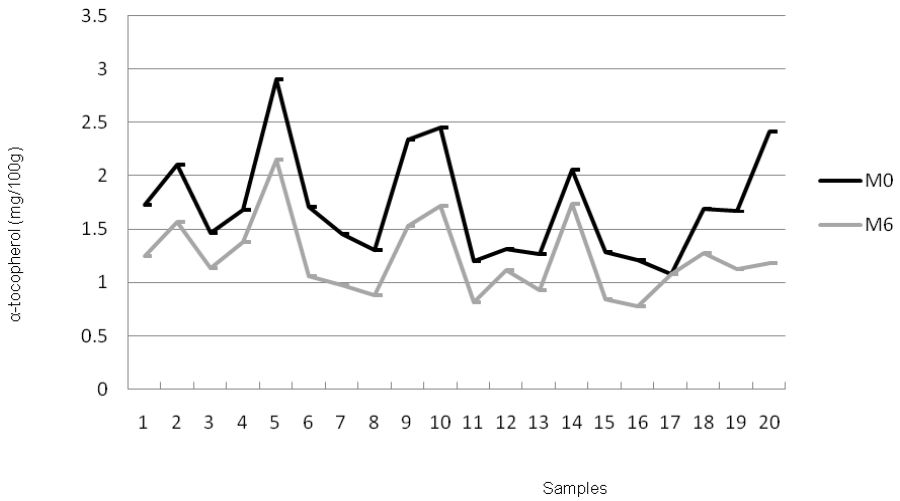

Figure 5. Variation of α-tocopherol contents (mg/100 g) after 6 months of storage.

Table 2. Evolution of β-carotene and α-tocopherol means from M0 to M6.

Parameter	Zero time (M0)	M6	Losses (%)	p
β-Carotene	57.38 ± 9.98	43.83 ± 7.83	24	0.0001
α-Tocopherol	1.72 ± 0.51	1.23 ± 0.36	28.49	0.0001

M0, First day of the analysis; M6, Sixth month of storage; p, Threshold of signification.

average loss of 70.4% of total carotenoids was reported working with *Ejumula* and *Kakamega* orange-fleshed sweet potato (OFSP) varieties in Uganda (Bechoff et al., 2010). Samples were conditioned in aluminous plastic bags which were not completely full (10 g/sachet). β-carotene and α-tocopherol are oxidative components, sensitive to oxygen from the air (Rastrelli et al., 2002; Burns et al., 2003; Ferreira et al., 2008). Due to their high degree of unsaturation, these components can extract or give up electrons, as a result, the radical anions and cations are likely to react with the oxygen retained in the conditioning bags and present antioxidative and oxidative properties under various conditions (McLaren and Frigg, 2001; Burns et al., 2003). Besides, the low contents of α-tocopherol in our samples, the key element for oxidative stability, suggest the vulnerability of spirulina powder to oxidation during the storage stage.

Conclusion

The present study is a contribution for the preservation of the nutritional value of spirulina during the semi-artisanal process of storage. The study revealed a variation of β-carotene and α-tocopherol contents from one sample to another and post-harvest losses. These results could explain the low nutritional value of the spirulina on the market. The commercialized spirulina would probably not have the same nutritional value and this nutritional value tends to decrease when stored over long periods of time.

The results from the present study suggest the use of this micro-seaweed as a dietary supplement in the shortest possible time after its harvest and its drying. Post-harvest losses of the nutritional value of spirulina can be due to several factors such as the packaging and the conditions of storage. These are important aspects to take into account at the production sites as well as throughout the marketing chain of the spirulina.

Conflict of interests

The authors did not declare any conflict of interest.

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