Characteristics of a Blue-Green Alga (*Spirulina platensis*) Preserved by Acidulation with Sulfuric Acid

S. Divakaran* and Eirik O. Duerr

Freshly harvested *Spirulina platensis* can be stabilized for extended storage by the addition of sulfuric acid (3% by weight). *Spirulina* preserved in this manner can be held at ambient temperature, without any deterioration in quality, for at least 20 days. Water that separates via gravity sedimentation from the acidulated *Spirulina* during storage can be siphoned off, thereby reducing total water content of the wet algae by as much as 33%. After the free water had been removed by centrifugation and the resultant material dried, the total ash content of acidulated algae was considerably lower than that of fresh. Acid-preserved *Spirulina* can be subjected to the extended periods of time required for sun drying without developing putrefactive odors or fungal blooms. Acidulated algae either dried in the sun or vacuum dried at low temperature had a higher pepsin digestibility index and a higher available lysine content than algae that was directly oven dried without prior acidulation.

Fresh *Spirulina platensis* contains 90% moisture. Unless it is refrigerated or oven dried within hours of harvest, autolysis and subsequent spoilage will proceed rapidly. The elimination or reduction of drying costs should contribute to improving the economic viability of using this material as a source of protein in animal feeds. Earlier work on the stabilization of cattle blood and offal via acidulation (Divakaran and Sawa, 1986) showed that these byproducts could be held at room temperature for as long as 10 days if blended with 3% (by weight) of 98% commercial sulfuric acid. This method of preserving offal and blood also allowed these materials to be subjected to the extended period of time required for sun drying. The same preservation technique was applied to *Spirulina*, to determine whether this method would have any specific advantage in further processing of *Spirulina*.

MATERIALS AND METHODS

S. platensis (UTEX 1928) was cultured in 125-m^2 raceways in Zarrouk's medium (Zarrouk, 1966) to an optical density of 0.7–0.8 and collected with a Sweco vibrating screen harvester. The alga was subsequently washed in fresh water to remove mineral salts entrained within the biomass. Lots weighing 10–30 kg were transferred to a 50-L-capacity stainless-steel ribbon blender and mixed with 3% by weight sulfuric acid (30 g of 98% acid/kg of *Spirulina*). The acid was previously diluted (50%) with water and allowed to cool to room temperature before blending to prevent violent reaction and charring. After 90–120 min of continuous blending, the stock was transferred to polyethylene buckets and left undisturbed at ambient temperature for 3 days. On the fourth day, the water that had separated and formed a lower layer was siphoned off. The upper layer of *Spirulina* (approximately two-thirds of the original volume) was further dewatered in 5-kg batches in a basket centrifuge at 1080*g* for 5–8 min. The dewatered *Spirulina* was removed and spread on wire mesh trays for sun drying. Sun drying took 5–8 days under normal sunny weather (25–28 °C). Acidulated samples from the same fresh batch were also dried in a vacuum oven at 60 °C (250–375 mmHg) for 4–6 h. Control samples from the same batch of *Spirulina* were dried on the day of harvest in a forward-flow convection oven at 100 °C without acidulation. The dried products from all treatments were ground in a Wiley mill to a coarse powder and passed through a sieve with 0.8-mm openings.

Experiments were conducted on the wet acidulated *Spirulina* to determine the length of storage possible and identify changes that take place during drying. A 1-kg batch of *Spirulina* blended with 30 g of diluted sulfuric acid was distributed in 100-g aliquots in 250-mL glass beakers covered with aluminum foil and stored at ambient temperature. One beaker was sampled every other day, until day 12, and again on day 20.

A portion of known weight from each 100-g sample was centrifuged for 20 min at 4080*g*, and the ratio of sediment to supernatant by weight was determined. Percent moisture in the sediment and percent solids in the supernatant were determined by heating a known weight of the sample at 98–100 °C for 4–5 h at 100 mmHg and obtaining the difference in weight according to AOAC methods (Williams, 1984). The same method was also used for determining moisture content of samples to express results on a moisture-free basis.

An estimation of protein quality between oven-dried, acidulated/sun-dried, and vacuum-dried *Spirulina* was made by conducting the following tests: (1) crude protein value; (2) pepsin digestibility (Williams, 1984); (3) available lysine (Kakade and Liener, 1969); (4) amino acid analysis.

Crude protein in the supernatant and sediment samples was determined with a Buchi 321 automatic Kjeldahl nitrogen instrument, according to AOAC methods. The ratio of protein to nonprotein nitrogen in the supernatant was determined on the 120-h sample by precipitating the proteins with trichloroacetic acid, followed by Kjeldahl nitrogen determination of the precipitated protein and supernatant solution (Mezincescu and Sazabo, 1936). Amino acid analyses were conducted on sun-dried and oven-dried *Spirulina* samples to identify any changes that may have taken place due to the drying method used. This analytical work was performed by Antech Laboratories (Corbett, OR).

The ash contents of fresh algae, acidulated algae with water separated by siphoning, and acidulated algae with water separated by siphoning followed by further centrifugal removal of water were estimated by ashing the samples at 600 °C for 6 h in a muffle furnace and determining percent ash on a moisture-free basis.

Differences in total fat content of the three samples of *Spirulina* (oven dried, vacuum dried, sun dried) were determined with a Soxhlet extraction apparatus at 30 extraction cycles, using a mixture of hexane and acetone in the ratio of 85:15.

Oceanic Institute, Makapuu Point, Waimanalo, Hawaii 96795.

0021-8561/87/1435-0568$01.50/0

Table I. Changes over Time in the Moisture and Crude Protein Contents of Acidulated *S. platensis* and Its Separated Liquid Fraction[a,b]

	wt % ea fraction[c]		moisture %		crude protein %[d]	
age of product, days	algal	liquid	algal	liquid	algal	liquid[e]
before acidulation						
0	62.5	37.5	85.2	99.1	58.5	4.9
after acidulation						
2	32.5	67.4	69.4	94.7	55.5	18.4
4	21.3	79.6	67.0	94.2	58.9	16.6
6	22.5	77.5	66.7	94.1	59.3	17.5
8	23.5	76.5	70.3	93.0	55.6	18.3
10	22.5	77.5	66.7	93.7	63.1	19.2
12	22.5	77.5	66.9	93.7	60.8	19.3
20	22.5	77.5	67.7	93.9	59.4	23.3

[a] Results given as means of triplicate experiments. [b] Fresh whole *Spirulina* contains 90.5% moisture and 63.1% crude protein. [c] Centrifuged at 4080*g* for 20 min. [d] Crude protein expressed on a moisture-free basis. [e] Crude protein in the trichlorocetic acid precipitated residue.

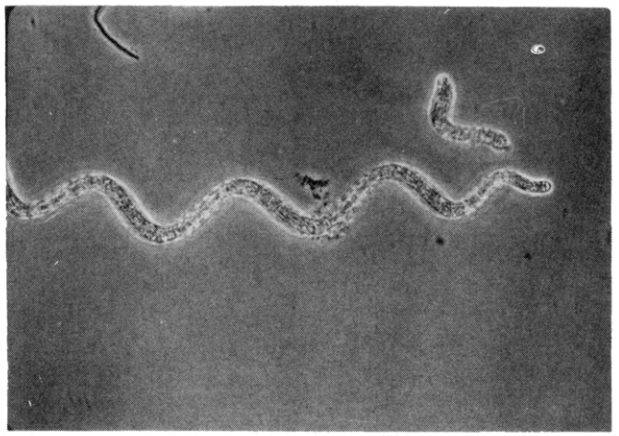

Figure 1. Fresh filaments of *S. platensis* (400× magnification). Cell diameters are approximately 6–8 μm. The spiral nature of the filament is evident from the alternating planes of focus.

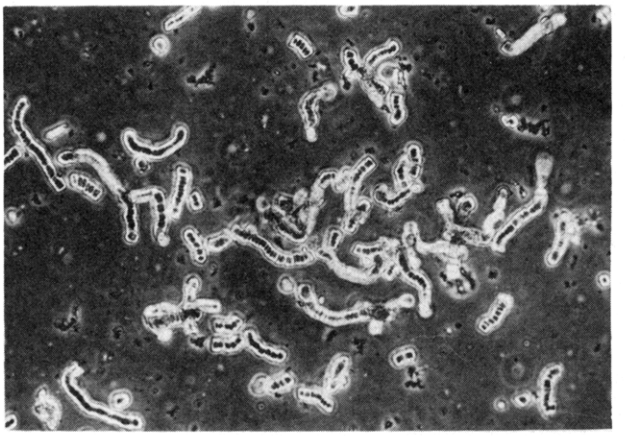

Figure 3. Nonacidulated, oven-dried *S. platensis* (400× magnification). The cells appear dry and shriveled and have lost the typical filamentous structure.

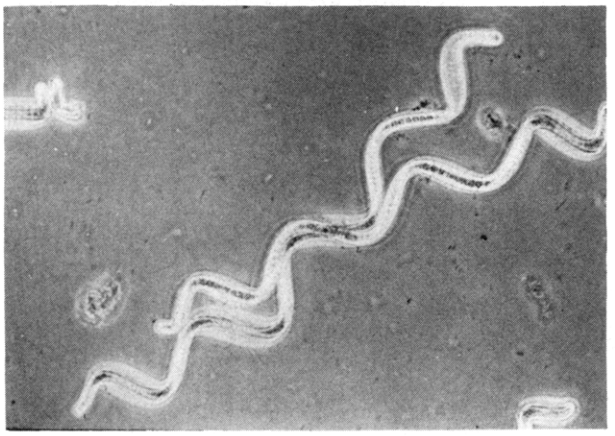

Figure 2. Wet, acidulated *S. platensis* (400× magnification). The filamentous structure is still intact although there is loss of intracellar differentiation and a clearer definition of cross walls between cells.

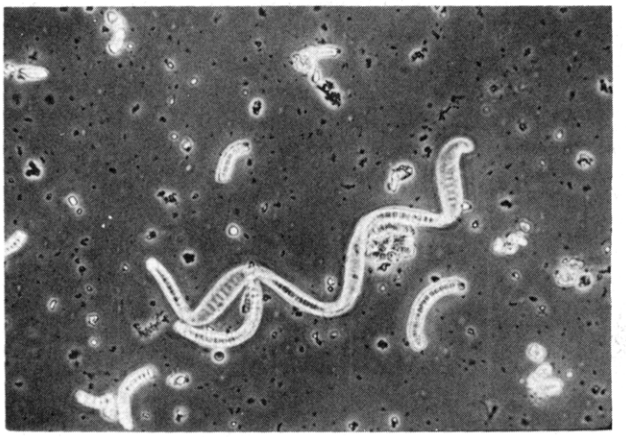

Figure 4. Acidulated, sun-dried *S. platensis* (400× magnification). Note that the filamentous structure is still intact although broken into fragments.

RESULTS AND DISCUSSION

Acidulation of fresh *Spirulina* with 3% by weight of 98% commercial sulfuric acid was found to preserve fresh *Spirulina* at ambient temperature without any putrefactive changes and minimal structural damage to the *Spirulina* filaments (Figures 1 and 2) when examined under the microscope during the 20-day storage period. The *Spirulina* filaments were found to be only partially destroyed in the sun-dried product, whereas oven drying destroyed the integrity of the cells (Figures 3 and 4). The acidulated stock changed from the initial pH 7.5–8.5 in freshly harvested *Spirulina* to pH 1.5–2.5 after acidulation and remained between pH 2.5 and 3.5 during the 20-day storage period.

The gravity separation of water from acidulated *Spirulina* is considered important because it allows the moisture level to be reduced without any expenditure of energy. Separation ranged from 30 to 35% of the total volume and did not show any greater separation even when the period of storage was extended beyond 3 days. The percent moisture in sediment and supernatant (Table I) remained fairly constant following the second day of storage. There was an increase in the total solids in the separated water from 0.9% for raw unacidulated *Spirulina* to 6% in the acidulated sample. There was no further loss

Table II. Proximate Analysis (%), Pepsin Digestibility (%), and Available Lysine Values for Oven-Dried and Acidulated Sun- and Vacuum-Dried *Spirulina*

method	crude protein[a]	fat[a]	ash[a]	pepsin digestibility	indigestible residue[b]	ALV,[c] res/mol
unprocessed	58.5	nd[d]	14.3	nd[d]	nd	nd
oven dried	58.8	6.4	14.5	62.5	82.5	28.2
sun dried	57.2	7.8	4.2	75.0	79.8	56.8
vacuum dried	57.6	7.4	5.4	72.1	78.1	53.4

[a] Percent on moisture-free basis. [b] Pepsin-indigestible residue = (percent crude protein in residue)/(percent crude protein in sample) × 100. [c] Available lysine value. [d] nd = not determined.

Table III. Amino Acid Composition (% Total Protein) of Oven-Dried *Spirulina* and Acidulated/Sun-Dried *Spirulina*

amino acid[a]	oven dried	sun dried
aspartic acid	12.98	13.10
threonine*	4.98	5.23
serine	5.33	4.71
glutamic acid	15.88	15.83
proline	13.18	4.44
glycine	5.61	4.46
alanine	7.39	7.07
valine*	5.82	5.23
methionine*	1.20	1.72
isoleucine*	3.58	4.98
leucine*	5.67	8.08
tyrosine	3.29	3.93
phenylalanine*	3.02	3.95
histidine*	3.62	6.10
lysine*	0.71	1.30
arginine*	6.28	8.41
NH_3	1.46	1.46

[a] Asterisks indicate essential amino acids.

of solids into the separated water after the second day.

An estimate of protein nitrogen in the trichloroacetic acid precipitated residue and the protein nitrogen in the supernatant by the Kjeldahl method indicated that supernatant had protein nitrogen in the range of 16.6–23.3% (Table I), the remaining being nonprotein nitrogen. This analysis also provided evidence that the loss of protein was minimal in the acidulation and dewatering process adopted for *Spirulina* processing.

An assessment of protein quality of *Spirulina* in terms of pepsin digestibility and available lysine for oven-dried, acidulated/sun-dried, and acidulated/vacuum-dried *Spirulina* is given in Table II. The three types of samples had similar total crude protein values. However, sun-dried and vacuum-dried *Spirulina* had higher pepsin digestibility and lower crude protein in the pepsin-indigestible residue than the oven-dried algae. The available lysine values (ALV) were higher for sun-dried and vacuum-dried *Spirulina*, compared to the oven-dried product.

The amino acid compositions of sun-dried and oven-dried *Spirulina* are reported in Table III. The essential amino acid tryptophan was not assayed but is expected to be reduced by the acidulation process. The content of essential amino acids was almost always higher in the sun-dried product than in the oven-dried product. Heat processing may have destroyed such amino acids in the oven-drying process. Proline content between the two processing methods, oven drying and sun drying, was definitely different. Proline is extremely soluble in water and in many marine eucaryotes and procaryotes serves as an osmoregulatory molecule (Duerr and Mitsui, 1982; Rudulier et al., 1984).

The ash content of *Spirulina* is influenced by culture method. *Spirulina* grown in Zarrouk's medium had an ash content of 14%, compared to *Spirulina* grown in manure-based media, which had ash contents from 18% to 28%. Acidulation and dewatering of the algae reduces the ash content of the dried product by 60–70% (Table II). Thus, acidulation does offer the advantage of providing a product of lower ash content than unprocessed oven-dried *Spirulina*. The reduction in ash content can be attributed to the higher solubility of most electrolytes present in the acid pH and their removal with the supernate via dewatering.

The effect that drying methods had on the total amount of fats extractable from *Spirulina* was determined via Soxhlet extraction of oven-dried, sun-dried, and vacuum-dried samples. The results (Table II) revealed that the drying method did not influence the recovery of extractable fats, which ranged from 6 to 8% on a moisture-free basis in all three cases.

From the experiments described above, it may be concluded that acidulation and sun drying do provide a dry *Spirulina* end product of higher protein nutritional quality. Acidulation of freshly harvested *Spirulina* allows it to be stored at ambient temperature, thus avoiding refrigeration costs. The gravity separation of a portion of the water also permits an additional energy savings. In addition, acidulation permits the product to be subjected to the extended period of time required for sun drying without spoilage. Vacuum drying at low temperature could be used as an alternative to sun drying when solar drying is difficult or not possible.

ACKNOWLEDGMENT

This research was supported by a grant from the U.S. Department of Agriculture/Agriculture Research Services (59-32U4-4-25).

Registry No. H_2SO_4, 7664-93-9.

LITERATURE CITED

Divakaran, S.; Sawa, T. *Agric. Wastes* **1986**, *17*, 67.
Duerr, E. O.; Mitsui, A. *CRC Handbook of Biosolar Resources*; CRC: Boca Raton, FL, 1982; Vol. 1, p 223.
Kakade, M. L.; Liener, I. E. *Anal. Biochem.* **1969**, *27*, 273.
Mezincescu, M. D.; Sazabo, F. *J. Biol. Chem.* **1936**, *115*, 131.
Rudulier, Le. D.; Storm, A. R.; Dandekar, A. M.; Smith, L. T.; Valentine, R. C. *Science (AAAS)* **1984**, *224*, 1064.
Williams, S. S., Ed. *Official Methods of Analysis of the Association of Analytical Chemists*, 14th ed.; AOAC: Washington, DC, 1984; p 152.
Zarrouk, C. C. These, de Doctorate d'etate es Sciences Appliquees (Faculte de Sciences de l'Universite de Paris), 1966.

Received for review September 8, 1986. Accepted March 23, 1987.