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Harvesting of *Aphanizomenon flos-aquae* Ralfs ex Born. & Flah. var. *flos-aquae* (Cyanobacteria) from Klamath Lake for human dietary use

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Abstract

In western cultures, certain cyanobacteria have been an accepted source of microalgal biomass for food for about 30 years, in particular *Spirulina (Arthrospira) platensis* and *S. maxima*. Beginning in the early 1980s, another species, *Aphanizomenon flos-aquae* was adopted for similar uses. This is harvested from Upper Klamath Lake, the largest freshwater lake system in Oregon. In 1998 the annual commercial production of *Aphanizomenon flos-aquae* was about 1×10^6 kg. Since this species is not cultured like *Spirulina* in outdoor ponds or raceways, it requires very different procedures for harvesting and processing. These are reviewed here and include extensive off-lake screens or on-lake barges, which dewater and concentrate the cells. Other procedures, such as those for removal of detritus and mineral materials, and those for monitoring and reducing the amounts of certain contaminant cyanobacteria, which can produce cyanotoxins, have also become important in quality control and marketing.

Introduction

Worldwide, algae have been a food source and a treatment for various physical ailments for thousands of years (Hoppe, 1979; Richmond, 1990). In coastal regions of the Far East recorded use of macroalgae (sea weed) as a food source began about 6000 BC, with evidence that many species were used for food and medical treatment by around 900 AD (Cannell, 1990). The Spanish recorded use of microalgae as a food source when they reported that the natives of Lake Texcoco collected cyanobacteria from the waters of the lake to make sun-dried cakes (Farrar, 1966; Ciferri, 1983). In current day Africa, local tribes harvest cyanobacteria in the Lake Chad region, primarily *Spirulina*, and also use it to make hard cakes, called dihé (Ciferri, 1983; Abdulqader et al., 2000). In some regions of Chad, people consume from to 9 to 13 g per meal, constituting 10 to 60% of the meal (Delpeuch et al., 1975). However, the longest recorded use of cyanobacteria as a food is the consumption of *Nostoc flagelliforme* in China, where there are records for some two thousand years and where it is still harvested on a large scale (Gao, 1998). Use of microalgae in Western culture began in the 1970s (Becker & Venkataraman, 1980). Most commercial producers of microalgae are located in the Asia-Pacific rim, where about 110 commercial producers of microalgae have an annual production capacity from 3–500 t. These cultivated microalgae include *Chlorella, Spirulina, Dunaliella, Nannochloropsis, Nitzschia, Crypthecodinium, Tetraselmis, Skeletonema, Isochrysis* and *Chaetoceros* (Lee, 1997).

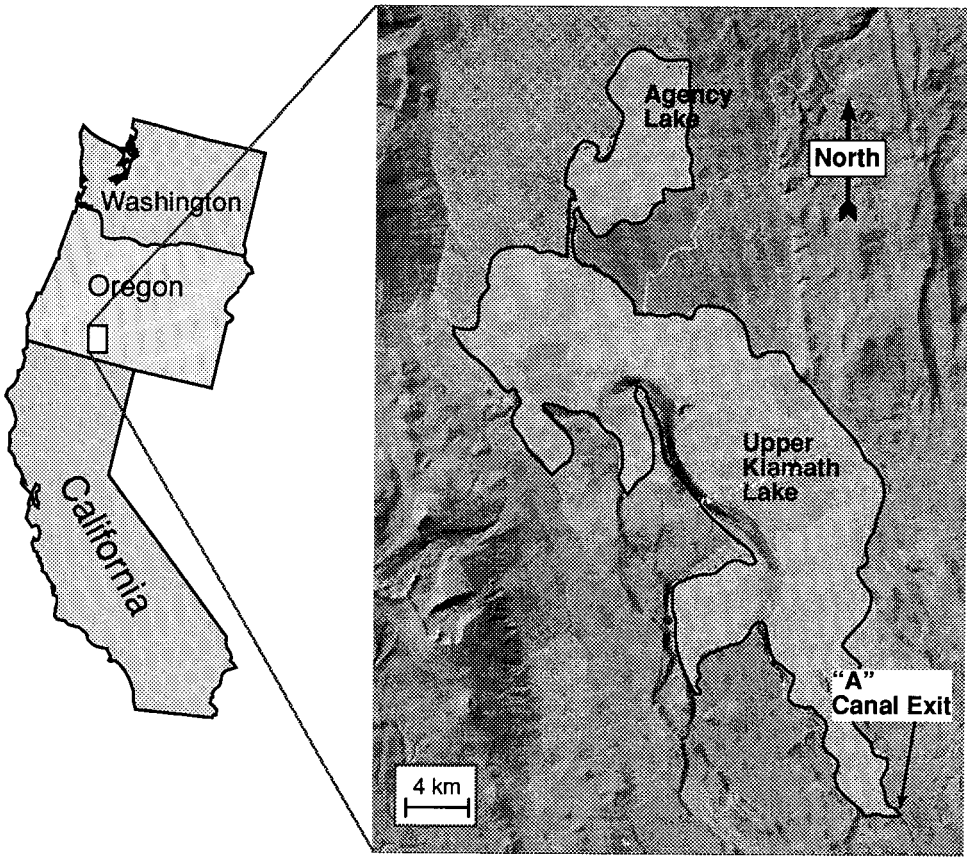

Figure 1. Upper Klamath Lake and contiguous Agency Lake located in southern Oregon, USA.

Within the cyanobacteria *Spirulina* (*Arthrospira*) *platensis* and *S. maxima*, have been commercially produced as a human and animal food supplement and food coloring for about thirty years (Ciferi & Tiboni, 1985; Belay et al., 1996). *Spirulina* is cultured in constructed outdoor ponds in Africa (Toerien & Grobbelaar, 1980), California, Hawaii, Thailand, China (Li & Qi, 1997), Taiwan (Soong, 1980) and India (Becker & Venkataraman, 1984). World production in 1995 was about 2×10^6 kg (Belay et al., 1996)

The newest cyanobacterium to be used as a food supplement is *Aphanizomenon flos-aquae*, the production of which differs significantly from *Spirulina*, because it is harvested from a natural lake rather than constructed ponds. Since the early 1980s this alga ('alga' will be used in this paper since this is the term used by all consumers of the product) has been harvested from Upper Klamath Lake, Oregon, and sold as a food and health food supplement. In 1998 the market for *Aph. flos-aquae* as a health food supplement was about $100 million with an annual production greater than 1×10^6 kg (d. wt). This paper reviews the harvesting and processing techniques currently in use on Klamath Lake to prepare this biomass plus quality control issues regarding the presence of cyanotoxins.

Klamath Lake ecosystem

Upper Klamath and contiguous Agency Lake is the largest freshwater lake system (surface area 324 km^2; mean shoreline length 161 km) in Oregon, draining a watershed of 9800 km^2 (Figure 1). Upper Klamath Lake is shallow, with a mean depth of 2.4 m, and is flanked by the Cascade Mountains to the west and the Great Basin to the east. Klamath Lake is fed by two major tributaries, the Williamson and Wood Rivers, as well as many smaller springs and stream inflows.

Historic accounts, dating back over 100 years, indicate that the lake has always been productive, supporting not only a high biomass of algae, but also fish,

waterfowl and predatory bird species. When ice was first collected from the lake (1906) it was reported to be green with algae. Lake suckers were so common that people used pitchforks to harvest them. Ospreys were reported in densities of up to 10 nests per square mile (Bortleson & Fretwell, 1993). Today the Klamath Basin is still home to the largest wintering congregation of bald eagles in the lower 48 states, and is the largest stopover for waterfowl in the Pacific flyway.

Klamath Lake has experienced increased eutrophication since its discovery by non-Native Americans (Wood et al., 1996; Kann, 1997). The lake's hydrology has been changed by water diversions in the upper watershed areas, and by the construction of a dam at the lake's outlet in 1921. Lake flushing flows, essential for nutrient export, have been altered and lake level has been reduced below pre-1921 levels. The lowered water levels have contributed to wind-induced resuspension of bed sediments, increasing the bioavailable nutrient levels in the lake (Laenen & LeTourneau, 1996). This factor, plus agricultural changes in the upper watershed, result in wetland inputs of 3 to 10 times the phosphorus (P) levels found in the Upper Klamath Basin tributaries (tributary inputs 65 μg L^{-1}) (Miller & Tash, 1967; Kann, 1997).

These changes to the lake and its watershed have undoubtedly contributed to the current conditions that allow *Aph. flos-aquae* to dominate consistently the phytoplankton from about June to October. The first report of *Aph. flos-aquae* was in 1933. By the late 1930s it was reported to be abundant, but not dominant, and by the mid 1950s it was dominant (Bortleson & Fretwell, 1993; Phinney & Peek, 1961). Records of algal blooms over the past 7 years show that *Aph. flos-aquae* is the dominant phytoplankton species in Upper Klamath Lake (Figure 2). Typically, blooms in Klamath Lake start in March dominated by the diatom *Fragilaria* ($\sim$5 mg L^{-1}). From late May to early June *Aph. flos-aquae* starts to bloom (3–15 mg L^{-1}). During this time a small bloom of *Anabaena flos-aquae* may occur, constituting less than 1% of the biomass. *Microcystis aeruginosa* and *Coelosphaerium* often appear in July and persist into late fall. *Aph. flos-aquae* remains the dominant species until October or November reaching a biomass that can exceed 50 mg L^{-1}. *Aph. flos-aquae* blooms are often biphasic, with a first peak in late June to early July, and a second peak late in September to mid-October (Kann, 1997). Estimates of standing crop are few, but one done for mid-summers 1965 and 1966 sets the *Aph. flos-aquae* dominated biomass at about 30×10^6kg (Miller &

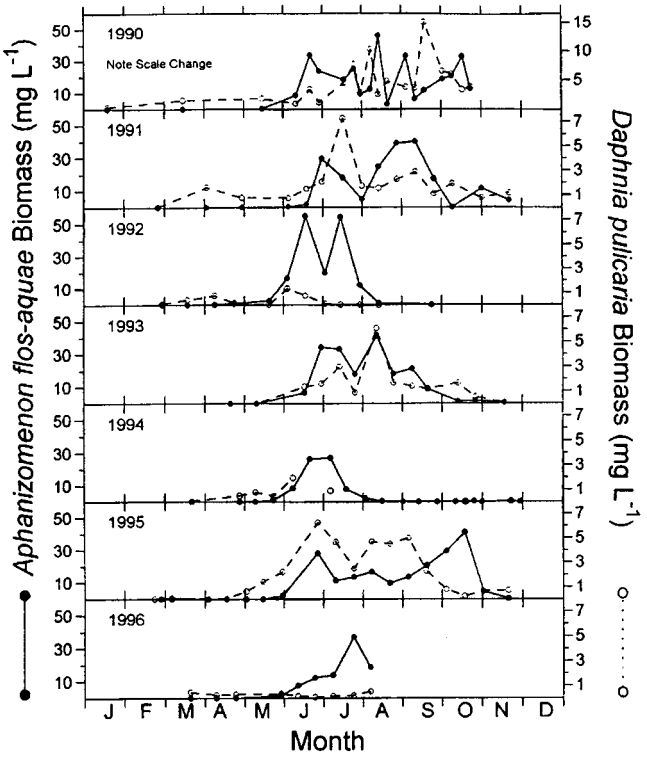

Figure 2. Lake-wide mean total biomass of *Aphanizomenon flos-aquae* (mg L^{-1} wet weight, solid line) and *Daphnia pulicaria* (mg L^{-1}, dashed line) in Upper Klamath Lake, Oregon, 1990–1996. Reproduced with the permission of J. Kann (Kann, 1997, p. 21).

Tash, 1967). Given these bloom dynamics harvesters of the lake's algal biomass emphasize harvesting from mid-June to mid-July and from September to mid-October.

Harvesting procedures

Two main harvesting methods are in use on Klamath Lake. The largest harvester removes *Aph. flos-aquae* after it passes out of the lake into an irrigation canal called the A-canal (Figure 1). From the canal a large series of screens is used to remove the biomass after which it is cleaned and dried (see further detail below). Three and sometimes four other operators harvest *Aph. flos-aquae* from the open lake. Here two harvesting techniques are used. Both use self powered barges. One type of barge has rotating screens that can be lowered just under the surface of the lake. The other type pumps the water plus alga from underneath the surface to screens on the barge. Since most of the alga floats within one meter of the surface these methods

can obtain a higher biomass than available at greater depths. Product harvested in this manner is held on the barges at ambient temperature or sometimes chilled to about 4 °C until the biomass is ferried to a shore processing plant. No data are currently available on the amount of product harvested by these open-lake operators.

Canal harvesting and processing

The following outlines the harvesting and processing of cyanobacteria by Cell Tech International, the first and largest operator on Klamath Lake. From 1980 until 1989, the company employed small-scale harvesting (a few thousand kg d. wt yr^{-1}) of *Aph. flos-aquae* by gravity flow from a hole drilled in an elevated concrete irrigation aqueduct leading from Klamath Lake. In 1989 this company secured rights to operate at their current location, which receives input from a section of Klamath Lake's aqueduct system known as the A-canal. This canal starts at the southern end of Klamath Lake (Figure 1) and travels southeast nearly 15 km before dividing into two separate aqueduct systems; the B-canal, which feeds irrigation water to the east, and the C-canal, which feeds irrigation water to southern Oregon and northern California. This spot, where the A-canal divides into B- and C-canals, is the location of the harvest facility (Figure 3). The choice of this location was influenced by the fact that there is an abandoned low head hydroelectric turbine facility with a 7-m drop at the head of the C-canal. This feature is used to gravity feed water onto harvesting screens.

Debris screens

At the head of the harvest facilities, the company maintains debris screens, which reduce flotsam and fish from entering the harvest facility (Figure 3). These debris screens resemble those frequently used on agricultural irrigation withdrawals around the Klamath basin, but are much larger. Front and rear screens, which range from 0.6 to 1.2 cm in mesh size along the front of the structure, are placed in vertical slots in front of the harvest facility inflows. As the front screens clog with debris, they are manually lifted and cleaned from the back with pressurized water. The rear screens are cleaned after the front screens are replaced. A total of 55 debris screens with approximately 150 m^2 of wetted surface area (Gutermuth et al., 1998) allow water to pass quickly and minimize the head differential across the structures. Since these debris screens are not located at the point of diversion from the Upper Klamath Lake (at the start of the A-canal), they are not required to meet government fisheries guidelines that apply to all 'fish screens'. Consequently, some small fish and debris do pass into the alga harvest facility where they need to be mechanically removed from the product.

Since some of these fish losses into the harvest facility include two species of sucker that are classed as federally endangered, special attention is given to the screening process. In 1996 work began with local Fish and Wildlife Service personnel on studies to address, entrapment of larval plus larger fish, velocity patterns around the debris screens, attraction of fish to harvest facility water releases, and changes in water quality that resulted from harvest procedures (Gutermuth et al., 1998).

Alga collecting screens

The first set of screens for collecting alga on the A-canal was constructed in 1990 and was designed to collect less than one-third of the C-canal flow (C-canal flow 20 m^3 sec^{-1}). In the fall of 1996, the harvest facility was expanded to handle the entire A-canal flow which can be up to 28.3 m^3 s^{-1}. A total of 46 screens were constructed with 8 screens located on the upper C-canal, 28 on the lower C-canal and 10 on the B-canal (Figure 3). On the C-canal screens, the elevation drop disperses the water over the screens, while on the B-canal, water is pumped over the screens. Screens are the site of initial de-watering, as the alga are retained on the surface, allowing the water to fall into the canal below.

The screens are made of nylon mesh with a dimension of 20 m^2 and lie over an expanded metal base at a slight angle to the horizontal. They operate much like a filter, with the water passing through and the alga remaining on top for collection. A manually operated water spray assembly that is located atop and across each screen moves the collected biomass to a trough where it is pumped to a secondary vibrating filter screen. This vibrating screen removes any unwanted material that might have escaped the initial canal debris screens. This screen also collects small crustaceans such as *Daphnia* (Figure 2), which are used as a commercial food product for the aquacul-

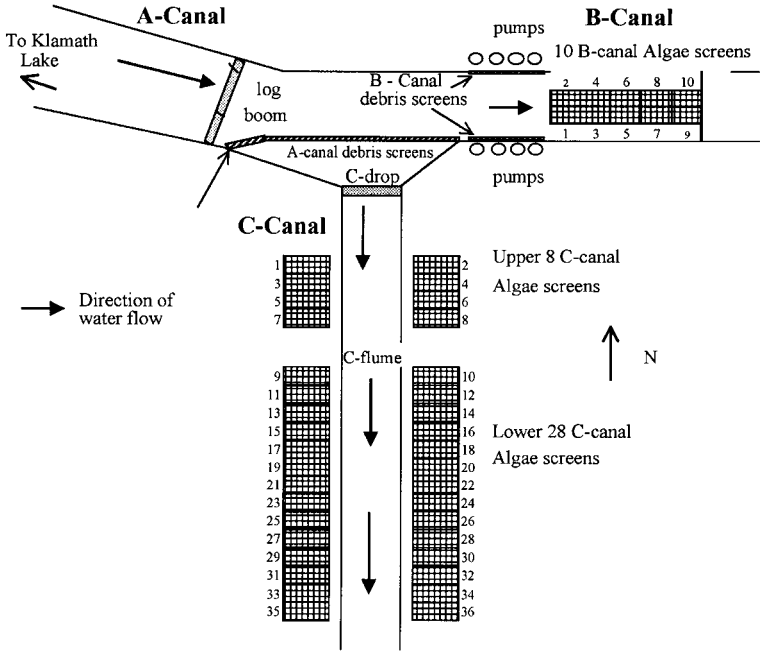

Figure 3. Schematic representation of Cell Tech's harvest site. Water flows down the A-canal to split into the B and C canals. In the C-canal, water is gravity fed over a series of screens. In the B-canal, water is pumped over a series of screens. At both locations, water is initially screened to restrict entrance of fish and debris into the site. Algal biomass collected at both sites flows into a trough and is pumped to a horizontal centrifuge for further processing.

ture industry. After passing through these vibrating screens, the alga slurry is approximately 0.1% solids.

Biomass of *Aph. flos-aquae* at the harvest site is highly variable, but monitoring the biomass in the lake and at the harvest site allows more efficient use of the harvest facilities. Flow rate in the canal is seasonally adjusted and regulated by the Klamath Irrigation District. The decision to harvest and the capacity at which the harvesting is operated is determined by the flow of product past the harvest site. This product flow (mg min^{-1}) is calculated by combining water flow (L min^{-1}) and product biomass (mg L^{-1}), as measured upstream from the screens. Figure 4 depicts the variation in phytoplankton biomass and the product flow at the harvest site during the 1998 harvest season.

Processing and storage

The product passing through the vibrating screen is pumped to a series of three slow speed horizontal centrifuges, which remove small extraneous material. This processing stage employs the fact that *Aph. flos-aquae* has a specific gravity almost equal to water, allowing for the centrifugal separation of sand, silt, and any other light filth. The algal concentrate is then gravity-fed into a 1200-m^2 processing plant. At the entrance to the processing plant, the algae is first passed through a cylindrical stainless steel screen to reduce extraneous material that could potentially damage the centrifuge that is used to further dewater and concentrate the algae. This centrifuge is vertical and applies high G-force to separate cells and colonies, removing about 90% of the remaining water. At this stage the algal product is 6–7% solids. Once concentrated, the product is chilled to 2 °C and stored before being pumped to the freezers.

The freezers are 6.8-m^2 stainless steel vertical cylinders that are ammonia jacketed and maintain a wall surface temperature of –50 °C. Alga is spread on the stainless steel surface approximately 3 mm thick by a series of tubes, where it freezes within a few seconds. The frozen algae is then removed from the freezer drum surface with a scraper blade, forming frozen pieces of algae about 5 to 10 cm in size. The frozen product then falls from the drums into storage boxes. The boxes are filled to a weight of approximately 450 kg and are removed from under the flake freezer via an automated conveyor system. The boxes are then bar coded with the date, time and weight, and shipped

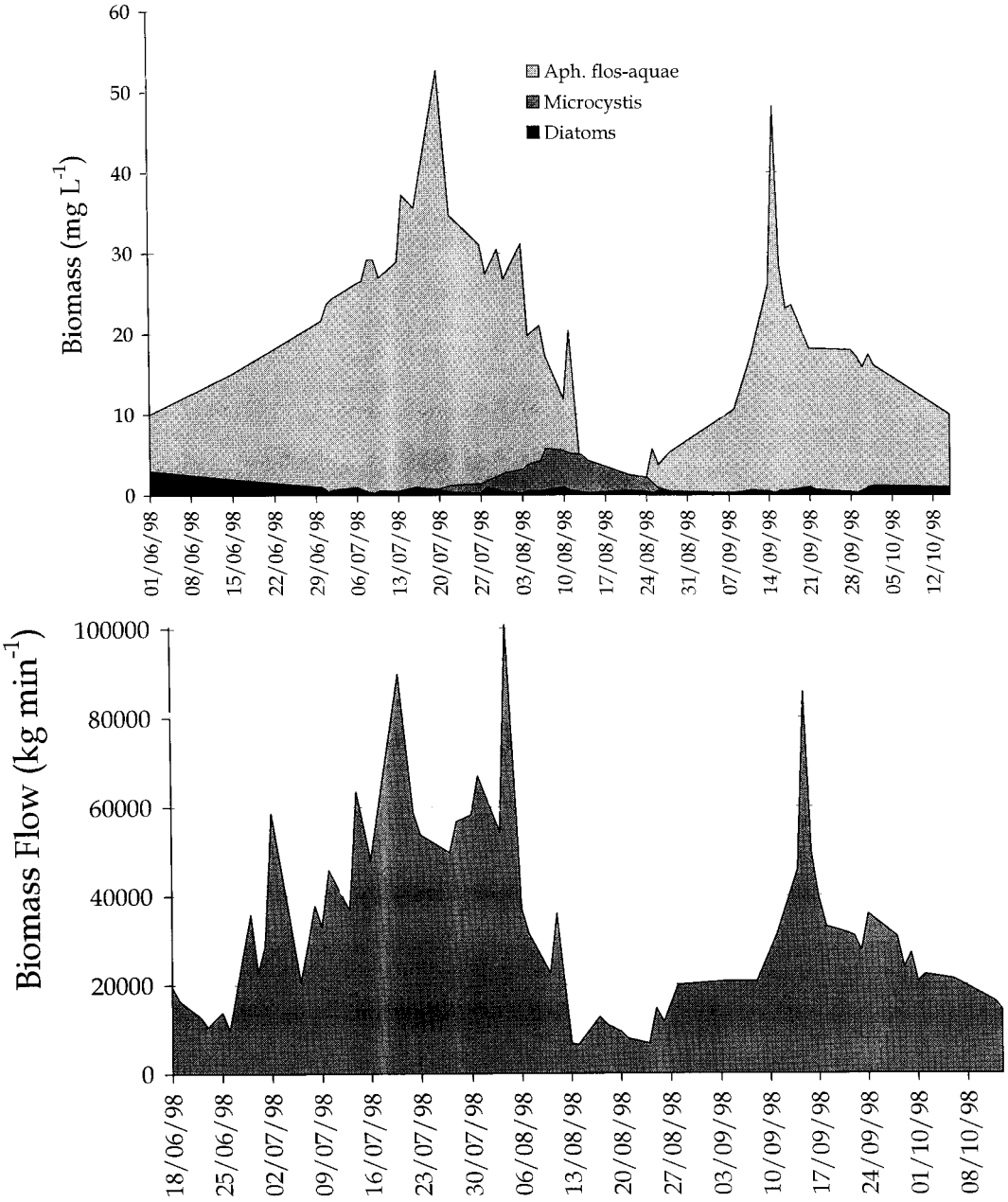

Figure 4. Top Biomass (mg L^{-1}) for the three primary phytoplankton at the harvest site on the C canal between June and October 1998. *Bottom* Flow of phytoplankton (kg min^{-1}) past the harvest site between June and October 1998.

to the freezer facility for storage. When needed, the frozen product is shipped to an external commercial freeze drying facility to be freeze dried into a powder containing 3 to 5% water content. This final product is processed into consumable products such as capsules or tablets.

Quality control procedures

In general all harvesters in the industry have a 'Quality Assurance/Quality Control' (QA/QC) program to perform various sampling regimes and tests during harvest and production to monitor product quality. Batches of powdered alga are subject to a series of tests to monitor safety and quality. Studies at the Cell Tech harvest site show that changes in the pattern of algal blooms on the lake take at least two to three days to show up at the harvest site. Therefore a batch normally consists of one day of harvest. Because of the potential for certain cyanobacteria to produce cyanotoxins (Carmichael, 1997) the dried biomass can be monitored using four different analysis for cyanotoxins: 1) Mouse bioassay for the detection of neurotoxins (saxitoxins (STXs),anatoxin-a). 2) Enzyme linked immunosorbant assay (ELISA) for detection of microcystins (hepatotoxins). 3) Spectrophotometric enzyme assay for presence of acetylcholinesterase inhibiting neurotoxin (anatoxin-a(s)). 4) Protein phosphatase inhibition assay (PPIA) for detection of microcystins (hepatotoxins).

A batch may range from 20 to 300 boxes (450 kg box^{-1}) of frozen alga. A one kilogram sample is taken from every box and labeled with the batch number and time. For each batch, Acceptance Sampling (Taylor, 1992) is used to determine the sample size that needs to be pulled for the composite sample to be representative of the whole batch. Frozen composite samples are freeze-dried, homogenized with a sifter, and sent for analysis. Batches are kept frozen until the test results are received. When a batch passes the tests, it is freeze-dried and then released for processing into the finished product.

Testing for neurotoxins

The scientific literature supporting neurotoxicity of Klamath Lake algal samples is inconclusive, and neurotoxins have never been found in phytoplankton from Klamath Lake during the past seven years of regular testing. However analysis for neurotoxicity is necessary because two strains of *Aphanizomenon* have been reported to produce neurotoxins (Mahmood & Carmichael, 1986; Rapala et al., 1993). One of these strains, NH-5 (producing saxitoxins, Mahmood & Carmichael, 1986), has been shown to be not the species *flos-aquae* (Li et al., in press), while the species in Klamath Lake is *flos-aquae* Ralfs ex Born. & Flah. var. flos-aquae (Li et al., in press). In addition, *Anabaena flos-aquae* can be found in small amounts early in the season prior to the start of harvesting, and this species has been reported from other areas of the USA and elsewhere to produce neurotoxins (saxitoxins (STXs)) and anatoxins (ANTXs). Therefore, the AOAC (Association of Official Analytical Chemists, 1990) mouse bioassay, which is more typically used to detect STXs in shellfish, is used to test for neurotoxic STXs and anatoxin-a in alga harvested from Klamath Lake. Another test used for anatoxin-a is a high performance liquid chromatography-fluorescence detection (HPLC-FL) assay (James et al., 1998). A colorimetric assay that determines inhibition of cholinesterase activity is used to test for anatoxin-a(s).

Mouse bioassay for the detection of saxitoxins and anatoxin-a

There is no standard method for detecting STXs in dried alga, so a modification of the AOAC (1990) method for STXs in shellfish is used. The limit of detection for STX, using this AOAC method, is 3 μg g^{-1} of dried algae. The quarantine limit where shellfish become a concern for human consumption is 80 μg STX per 100 g meat. It is generally believed that this limit assumes that 100 g of shellfish are consumed in a meal, equivalent to a possible intake of 80 μg STX. A human would have to eat nearly 27 g (108 capsules) of alga containing 3 μg g^{-1} (limit of detection) to reach this warning threshold. The lethal dose for humans is 6 to 12 times higher. The limit of detection for anatoxin-a is 5 μg and the LOAEL (Lowest observed adverse effect level) has been established at 100 μg kg^{-1} body weight. A human would have to eat nearly 1.4 kg of algae containing 5 μg g^{-1} (limit of detection) to reach a toxic level.

Anticholinesterase assay for anatoxin-a(s)

Another assay performed to monitor possible contamination by toxic *Anabaena flos-aquae* tests for the pos-

sible presence of the anticholinesterase toxin anatoxin-a(s). The method is based on a colorimetric assay for determination of cholinesterase activity. Mahmood & Carmichael (1987) and Matsunaga et al. (1989) adapted it for the detection of the anatoxin-a(s). Inhibition of the acetylcholinesterase enzyme is measured by a decrease in the color generated and monitored at 412 nm from the reaction of the enzyme and the substrate acetylthiocholine. This percent inhibition is compared against a standard curve of percent inhibition vs. log dose of purified anatoxin-a(s). All activities are compared against values obtained with enzyme plus no sample and sample plus no enzyme.

Testing for hepatotoxins

Microcystis and *Oscillatoria* are normal components of the cyanobacterial populations in Klamath Lake. Both genera can produce microcystins whose ingestion in high concentration can lead to acute liver damage and lethality. Microcystin content and activity is determined using an ELISA and PPIA, respectively. The State of Oregon's Department of Agriculture has set a safe limit of 1 μg microcystin per g dry weight of product. This level was set based upon an evaluation of the safe level that was recently established as a guideline value for drinking water by a panel working with the World Health Organization. This value for drinking water is 1 μg L^{-1} for a person drinking an average of 2 L day^{-1} (Chorus & Bartram, 1999). When the State of Oregon set this regulatory level, they left the details for analyses and quality control up to industry. Most of the harvesters have adopted an immunoassay and/or enzyme assay to measure levels of microcystin.

ELISA and PPIA for microcystins

The immunoassay (ELISA) method is based on the polyclonal antibody method described by Chu et al. (1989, 1990) and the PPIA as given by An & Carmichael (1994). The level of sensitivity for microcystin using these methods is about 0.05 ng.

Microcystins have been shown to be specific and potent inhibitors of protein phosphatases 1 and 2A (PP1 and PP2A). Inhibition of these enzymes correlates with these toxins' ability to promote tumors and to provoke liver damage. Therefore, the PPIA is useful in combination with the ELISA (which tests for presence of microcystins – not all of which are bioactive) as an activity assay (to measure actual toxic effect). The assay is about 1000 times more sensitive than the HPLC or mouse bioassay. The assay of PP activity is done by measuring the rate of color formation from the liberation of P-nitrophenol from P-nitrophenol phosphate using a microplate reader. Results of monitoring for microcystins using these two methods are given in Figure 5. The correlation coefficient for comparison of ELISA with PPIA is given in Figure 6.

Discussion

The remarkably stable abundance and highly available biomass of *Aph. flos-aquae* in Klamath Lake has provided the basis for a viable commercial harvesting of this cyanobacterium. The availability of such a large amount of biomass (standing summer crops $> 10^6$ kg), which is effectively unlimited during the growing season and free of any production related expenses, would seem to give *Aphanizomenon* a distinct economic advantage in the market. This is especially true when it is considered that *Aph. flos-aquae* can be marketed as 'wild harvested', which is a distinct marketing advantage in the present health food market. However, the logistics and quality control issues involved in harvesting and processing a safe alga biomass for natural food products presents more obstacles than the *Spirulina* industry encounters. Because the location for producing microalgal biomass can be situated wherever optimum conditions exist for maximizing yield and minimizing quality control issues, these issues are less troublesome for this industry. Although there is no cost-benefit analysis comparing the advantages of 'pond grown' vs. 'wild harvested', it would seem that there are advantages and disadvantages of each.

For harvesters of *Aph. flos-aquae*, the biggest expense has been the development of harvesting and processing procedures to assure a high quality product that meets all guidelines and regulations regarding agricultural products for human consumption. While there are no standard methods for analysis of possible cyanotoxins in microalgal biomass, the industry is moving to incorporate scientifically acceptable and accurate methods for detection of all relevant cyanotoxins. Based on the cyanobacterial genera and species present during the harvest season, certain neurotoxins and hepatotoxins could be a quality control issue

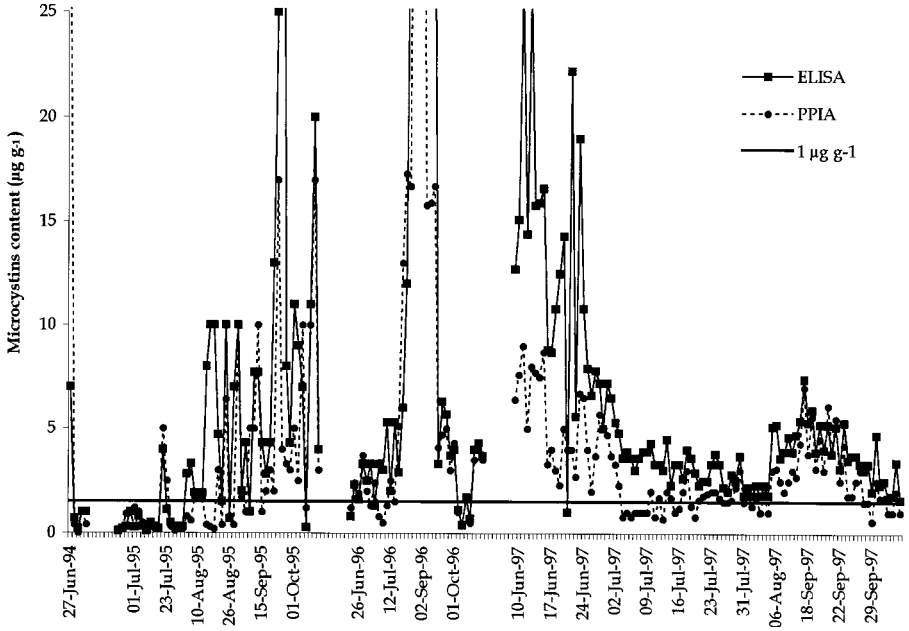

Figure 5. Microcystin content of algal biomass at the harvest site between 1994 and 1998 as measured by ELISA and PPIA. The State of Oregon's maximum acceptable concentration (MAC) of 1 μg g^{-1} is marked by a solid line on the graph. Batches containing microcystin levels below this MAC are used for making products for human consumption.

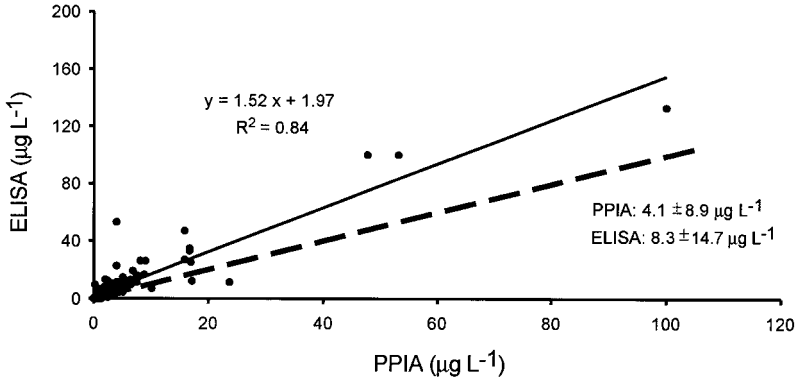

Figure 6. Correlation of the ELISA vs. the PPIA assay for measurement of microcystins. The PPIA results are consistently lower than those obtained by ELISA, with a correlation coefficient of 0.84. Microcystin-LR is the standard used for calibration of the plate assay. Dotted line shows where the ELISA and the PPIA would give identical results.

in using *Aph. flos-aquae*. While the literature reports that *Aphanizomenon* can produce neurotoxins including saxitoxins and anatoxin-a (Carmichael, 1997) all tests on Klamath Lake algae since 1992 have failed to detect any cyanobacterial neurotoxins when examined by mouse bioassay, HPLC, or mass spectrometry (Anderson & Carmichael, unpublished data). The only cyanotoxin found in the lake phytoplankton, during this testing period, is microcystin. Comparison of microcystin levels with phytoplankton composition supports *Microcystis* (Carmichael et al., unpublished) being the producer of microcystin in Klamath Lake phytoplankton. To date there are no confirmed reports of *Aph. flos-aquae* producing microcystin.

When the State of Oregon Department of Agriculture set a limit on microcystin content in algal products from Klamath Lake, they did not define a standard method for monitoring microcystin content. Since the HPLC methods available in the literature are not designed to routinely and rapidly analyze levels at or below 1 μg g^{-1}, the ELISA method (An & Carmichael, 1994) has been the primary method of screening

algae for microcystins. Although the ELISA method has been used successfully by others for microcystin analysis (Ueno et al., 1996), none of the ELISA antibodies have been tested for cross reactivity on all of the known microcystins (about 60). For this reason, and to test the correlation between ELISA as a chemical test and an activity assay, harvesters have tested microcystin content using the protein phosphatase inhibition assay (PPIA). This activity assay measures inhibition of the protein phosphatase enzyme, and if all inhibition found in a sample is due to microcystin, then there should be a good correlation between the two assays. Figure 6 shows that this is the case for microcystins present in Klamath Lake cyanobacteria. This figure also shows that the ELISA test gives consistently higher values of microcystin content than the PPIA. This suggests that the combined PP inhibition activity (i.e. toxicity) of microcystins present is less than the value for microcystin content using the ELISA, when microcystin-LR is used as the standard to calibrate both methods. This means that the use of the ELISA to monitor and regulate algal product can overestimate the risk to consumers from microcystin. However, because of the uncertainty of the long-term use risk from microcystins it also serves as an added safety factor, when this method is used to determine whether the product is acceptable for human and animal use.

Biomass from *Aphanizomenon flos-aquae* is a relatively new source of food and food nutritional supplements. In order to ensure that the market remains strong for these products several areas must be addressed.

1. Quality assurance must be maintained and any new issue that could affect safety and decrease quality must be addressed quickly and openly.
2. Regulatory officials and consumers must be educated with the facts regarding microalgae biomass and its potential benefits, compared to other sources of healthy food products and food supplements. This will help to minimize the need for government regulation of the industry and assure the highest level possible of consumer confidence.
3. New products and uses for microalgae biomass need to be developed if the markets for this new food are to expand beyond their present limited use.

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References

Abdulqader G, Barsanti L, Tredici MR (2000) Dihé: an ancient food in a forgotten world. J. appl. Phycol. 12:

An JS, Carmichael WW (1994) Use of a colorimetric protein phosphatase inhibition assay and enzyme linked immunosorbent assay for the study of microcystins and nodularins. Toxicon 32: 1495–1507.

Association of Official Analytical Chemists (1990) Official Methods of Analysis, 15th edn, revised. AOAC, Washington, DC, pp. 881–882.

Becker EW, Venkataraman LV (1984) Production and utilization of the blue-green alga *Spirulina* in India. Biomass 4: 105.

Becker EW, Venkataraman LV (1980) Production and Processing of Algae in Pilot Plant Scale Experiences of the Indo-German Project. In Shelef G, Soeder CJ (eds), Algal Biomass, Production and Use, Elsevier/North Holland Biomedical Press, Amsterdam, pp. 35–50.

Belay A, Kato T, Ota Y (1996) *Spirulina* (*Arthrospira)*: potential application as an animal feed supplement. J. appl. Phycol. 8: 303–311.

Bortleson GC, Fretwell MO (1993) A Review of Possible Causes of Nutrient Enrichment and Decline of Endangered Sucker Populations in Upper Klamath Lake, Oregon. U.S. Geological Survey Water-Resources Investigations Report 93-4087, Portland, 24 pp.

Cannell RJP (1990) Algal biotechnology. Appl. biochem. Biotech. 26: 85–105.

Carmichael WW (1997) The cyanotoxins. In Callow JA (ed.), Advances in Botanical Research, vol. 37. Academic Press, London, pp. 211–256.

Chorus I, Bartram J (eds) (1999) Toxic Cyanobacteria in Water – A Guide to Their Public Health Consequences, Monitoring and Management. E & FN Spon, London, 416 pp.

Chu FS, Huang X, Wei, RO (1990) Enzyme-linked immunosorbent assay for microcystins in blue-green algal blooms. J. Ass. off. Analyt. Chem. 73: 451–456.

Chu FS, Huang X, Wei RO, Carmichael WW (1989) Production and characterization of antibodies against microcystins. Appl. environ. Microbiol. 55: 1928–1933.

Ciferri O (1983) *Spirulina*, the edible microorganism. Microbiol. Revs 47 (December): 551–578.

Ciferri O, Tiboni O (1985) The biochemistry and industrial potential of *Spirulina*. Ann. rev. Microbiol. 39: 503–526.

Delpeuch F, Joseph A, Cavelier C (1975) Consumption as food and nutritional composition of blue-green algae among populations in the Kanem region of Chad. Ann. Nutr. Aliment. 29: 497–516.

Farrar WV (1966) Tecuitlatl: A glimpse of aztec food technology. Nature 5047: 341–342.

Gao K (1998) Chinese studies on the edible blue-green alga, *Nostoc flagelliforme*: a review. J. appl. Phycol. 10: 37–49.

Gutermuth FB, Beckstrand D, Watson C (1998) New Earth harvest site monitoring: 1996–1997. New Earth/Cell Tech Research and Development Dept., final technical report to the U.S. Fish and Wildlife Service, 120 pp.

Hoppe HA (1979) Marine algae and their products and constitutents in pharmacy. In Hoppe HA, Levring T, Tanaka Y (eds), Marine Algae in Pharmaceutical Science. Walter de Gruyter Publs, New York, pp. 25–119.

James KJ, Furey A, Sherlock IR, Stack MA, Twohig M, Caudwell FB, Skulberg OM (1998) Sensitive determination of anatoxin-a, homoanatoxin-a and their degradation products by liquid chromatography with fluorimetric detection. J. Chromatography A 798: 147–157.

Kann J (1997) Ecology and water quality dynamics of shallow hypereutrophic lake dominated by cyanobacteria (*Aphanizomenon flos-aquae*). PhD Dissertation, Univ. North Carolina, Chapel Hill, 110 pp.

Laenen A, LeTourneau AP (1996) Upper Klamath basin nutrient-loading study – Estimate of wind-induced resuspension of bed sediment during periods of low lake elevation. U.S. Geological Survey Open-File Report 95-414, Portland, Oregon, 11 pp.

Lee YK (1997) Commercial production of microalgae in the Asia-Pacific rim. J. appl. Phycol. 9: 403–411.

Li D-M, Qi Y-Z (1997) *Spirulina* industry in China: Present status and future prospects. J. appl. Phycol. 9: 25–28.

Li R-H, Carmichael WW, Liu Y-D, Watanabe MM (2000) Taxonomic re-evaluation of *Aphanizomenon flos-aquae* NH-5 based on morphology and 16S rRNA gene sequences. Hydrobiologia (in press).

Mahmood NA, Carmichael WW (1987) Anatoxin-a(s), an anticholinesterase from the cyanobacterium *Anabaena flos-aquae* NRC-525-17. Toxicon 25: 1221–1227.

Matsunaga S, Moore RE, Niemczura WP, Carmichael WW (1989) Anatoxin-a(s) a potent anticholinesterase from *Anabaena flos-aquae*. J. am. chem. Soc. 111: 8021–8023.

Miller WE, Tash JC (1967) Interim Report Upper Klamath Lake Studies Oregon. Federal Water Pollution Control Administration, Corvallis, 37 pp.

Phinney HK, Peek CA (1961) Klamath Lake, an instance of natural enrichment. Transactions of the Seminar on Algae and Metropolitan Wastes, April 27–29 1960, U.S. Public Health Service: 22–27.

Rapala J, Sivonen K, Lyra C, Niemelä SI (1993) Anatoxin-a concentration in *Anabaena* and *Aphanizomenon* at different environmental conditions and comparison of growth by toxic and non-toxic *Anabaena* strains, a laboratory study. Appl. environ. Microbiol. 64: 2206–2212.

Richmond A (1990) Handbook of Microalgal Mass Culture. CRC Press, Boca Raton, Florida, 528 pp.

Soong P (1980) Production and Development of *Chlorella* and *Spirulina* in Taiwan. In Shelef G, Soeder CJ (eds), Algal Biomass, Production and Use. Elsevier/North Holland Biomedical Press, Amsterdam, pp. 97–113.

Taylor WA (1992) Acceptance Sampling, Taylor Enterprises Inc., Lake Villa, Illinois, Ch. 8, pp. 171–190.

Toerien DF, Grobbelaar JU (1980) Algal mass cultivation experiments in South Africa, In Shelef G, Soeder CJ (eds), Algae Biomass, Production and Use, Elsevier/North Holland Biomedical Press, Amsterdam, pp. 73–80.

Ueno Y, Nagata S, Tsutsumi T, Hasegawa A, Watanabe MF, Park HD, Chen GC, Chen G, Yu SZ (1996) Detection of microcystins, a blue-green algal hepatotoxin, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay. Carcinogenesis 17: 1317–1321.

Wood TM, Fuhrer GJ, Morace JL (1996) Relation between selected water-quality variables and lake level in upper Klamath and Agency Lakes, Oregon. U.S. Geological Survey Water-Resources Investigations Report 96-4079, Portland, 57 pp.