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Volatile Organic Composition of Fresh and Dried *Spirulina platensis* from Kakamega, Kenya

Wekesa N. Justus[1] & Lilechi D. Baraza[2*]

[1]Faculty of Science, Kibabii University College, P.O Box 1699-50200, Bungoma, Kenya.

[2]Faculty of Science, Masinde Muliro University of Science and Technology, P.O. Box 190-50100, Kakamega, Kenya.

* Corresponding author email: bdanstone@yahoo.com

Abstract

The soxhlet hexane extracts of cultivated *Spirulina platensis* were analysed by Gas Chromatography coupled with Mass Spectrometry (GC/MS). On comparison between fresh and dried *S. platensis* samples, we found that the high number of metabolites were recorded in the fresh extract. Studies on the fresh and dry extracts resulted in the identification of thirty eight major compounds from fresh sample and twenty five compounds from dried sample. The metabolites were classified as fatty acids and their esters, alcohols, benzene derivatives, alkanes, alkanoic acids, esters, sterol phytol, and vitamin E. The major volatile organic compounds for fresh S. platensis extract were p-Xylene (24.584%), heptadecane (18.362%) and phenylethylalcohol (12.035%), while the major volatile organic compounds for dried *S. platensis* extract were Z-11-Hexadecenoic acid, (16.991%), Methyllinoleate (12.243%) and Ethylhexadecanoate (8.1363%). We also identified the presence of one sterol phytol in fresh extract that has earlier been documented. Our study is therefore the first report on the chemical composition of the non-polar extracts of edible *Spirulina platensis* grown in Kakamega, Western part of Kenya.

Keywords: *Spirulina platensis*, Chemical profile, volatiles, GC-MS analysis.

1. Introduction

Spirulina is a very nutritious and edible blue green micro algae which is an excellent source of protein, beta-carotene, B Vitamins and minerals like iron. In addition, *Spirulina* has a 62% amino acid content and contains all essential fatty acids and eight amino acids required for complete nutrition and other medicinal properties.[1, 2, 3, 4, 5, 6] *Spirulina* species are well distributed in soda lakes in Kenya and also grown as a nutritional supplement. Some of the species include *Spirulina platensis, Arthrospira maxima,* and *Spirulina fusiformis*. They also possess some aroma on or after harvest. It is envisaged that some of these volatiles could be curative or could contain some biological functions. Several studies have shown that *Spirulina platensis* or its extracts could prevent or inhibit bacterial and fungal diseases in humans.[7,8] Recent investigations have shown that *Spirulina* species are composed of lipid classes, including rare fatty acids, e.g tocopherols. [9, 10, 11, 12] This species has shown to have immuno-promoting effects [13] and inhibition of the replication of viruses such as herpes simplex and H.I.V-I.[14] However, to the best of our knowledge, there is no information available in the literature concerning the phytochemical composition of the Kenyan cultivated *Spirulina platensis*, despite the fact that it has been used as a source of food in this region. We therefore report the results of the GC-MS analyses of volatile compounds, identified from this species.

2. MATERIAL AND METHODS

2.1. *Spirulina platensis* Material

The fresh sample of wet *S. platensis* was obtained from MMUST in Kakamega County, Western Kenya. The location in Kakamega is at altitude of 1535 metres. The algal samples of *S. platensis* were identified based on morphological identification by Botanist Aggrey Osogo Keya of the Department of Biology, Masinde Muliro University of Science and Technology (Kenya). The dried sample was prepared by air-drying part of the same amount of the wet collected S. platensis in the shade at room temperature to form pieces of flakes. The dried sample was further broken into small pieces, placed in a blender and crushed to powdered material then stored in brown paper bags in a dark, dry place at room temperature until extraction.

2.2. Extraction

Each of the samples (wet and dried) was subjected to soxhlet extraction in hexane for 12 hours. 5 g of each sample was placed inside a thimble made from thick filter paper which was loaded into the main chamber of the soxhlet extractor separately. The soxhlet extractor was then equipped with the condenser. The hexane solvent in the flask was heated to 70^{o}C to vapourise and then condensed back down into the chamber housing the solid *Spirulina* material. The chamber containing the *Spirulina* was slowly filled with hexane and the oil in the Spirulina dissolved in the warm hexane. When the soxhlet chamber was almost full, the chamber was automatically emptied by a siphon side arm, with the solvent running back down the distillation. This was repeated six more times and afterwards the solvent that contained each of the resulting extract was vaporized by a gentle stream of nitrogen gas through the solution and kept at the fridge before being subjected to GC-MS analysis.

2.3 Analysis and Identification of volatile compounds

Both qualitative and quantitative characteristics of the extract were studied using Gas-chromatography-Mass Spectrometry (GC-MS) technique.[15] The constituents of the hexane oils were identified by analysis of their mass spectra, direct comparison of their mass spectra to the Wiley NBS and NIST databases or library of mass spectra and co-injection with authentic standards on the GC.

The GC analyses were performed with a Hewlett Packard HP 5890A Gas Chromatography equipped with a flame ionization detector (at 230 °C). A fused silica capillary column (Hewlett Packard, 50 m x 0.22 mm x 0.33 mm CD) coated with methyl silicon (0.3 µm film thickness) was used with nitrogen as the carrier gas. All GC analyses were performed in the splitless mode with the injector temperature at 270 °C. The oven temperature was programmed from 60 °C isothermal for 7 min, to 120 °C at 5 °C per min, then to 180 °C at 10 °C per min and finally to 220 °C at 20 °C per min, where it was maintained for l0 min. Peak areas were calculated using a Hewlett Packard 3393 B series integrator and together with their GC retention times, compared to those of authentic samples.

3. Results and Discussion

The mixture of volatile components were obtained from the fresh and dried cultivated Spirulina platensis and analyzed as described in the experimental section. The results obtained are outlined in Table 1. Fig 1 and 2 indicates the GC profile, giving the abundance of these compounds. The groups of compounds in the volatile fractions from both fresh and dry samples were characterized as fatty acids and their esters, alcohol, benzene derivatives, alkanes, alkanoic acids, sterols, and vitamins. The major chemical constituents in fresh sample

were p-xylene (24.584%), heptadecane (18.362%), phenylethyl alcohol (12.035%), Ethylbenzene (5.912%), Methyl linoleate (4.432%), Phytol (2.909%), tricyclo [5.3.0.0(3,9)] decane (2.657%), n-hexadecanoic acid (2.187%) and hexadecane (1.092%). The major chemical constituents which were detected in dry sample were Z-11-Hexadecenoic acid (29.99%), Methyl linoleate (22.48%) , Phytol (12.49%), methyl hexadecanoate (4.99%), tricyclo [5.3.0.0(3,9)] decane (4.98%), heptadecane (1.95%), n-hexadecanoic acid (1.56%), tricosane (1.56%) and 4,7,7-trimethylbicyclo[2.2.1] heptan-2-one (1.53%).

Twelve esters were recorded in the fresh sample and fluctuated between (0.0072-0.3851%), while dried sample recorded seven esters reached to higher percentage mass fractions (0.0434–12.243%) (supporting information, Table 1). The esters enhance the different fruit fragrance such as butyl acetate (banana or apple), methyl hexadecanoate (oily waxy fatty orris), phytol acetate (fruity balsamic) and methyl butanoate (pineapple). Methyl linoleate, was found in considerable amounts in wet S. platensis. Methyl linoleate serves as a precursor of (E, Z)-3, 5-octadien-2-one and is one of the most odor active compounds which contributes to the melon-like odor. [16]

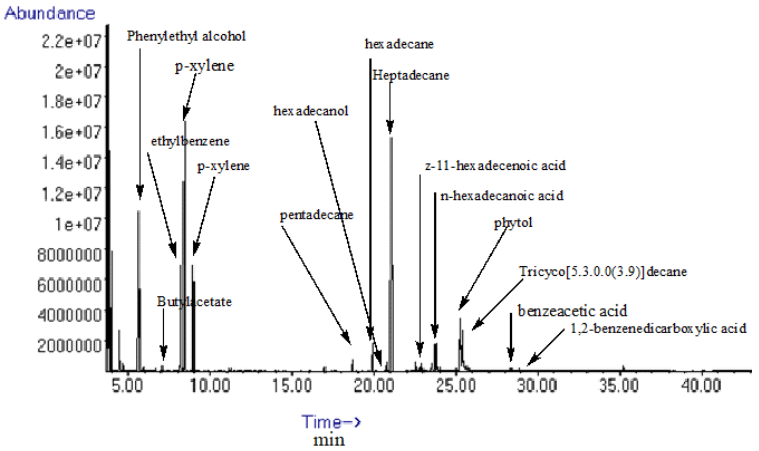

Figure 1: GC- MS profile for wet Spirulina soxhlet hexane extract (Absorbance vs time in min)

Many compounds previously in the wet sample were not observed in the dried sample. The dried *S. Platensis* yielded the GC profile in Fig 2 that was a clear indicator of the disappearance of the major compounds p-xylene and parts of phenylethyl alcohol at retention time 8.4-8.9 mins and 5.6 mins respectively. Hazardous compounds like p-xylene, which are characterized as hazardous and carcinogenic can easily be separated by distillation or temperature controlled drying, a

method that our research group has already adopted. However, it is not known why these species produce this compound, possibly as a defense mechanism from sea animals or pests. [17]

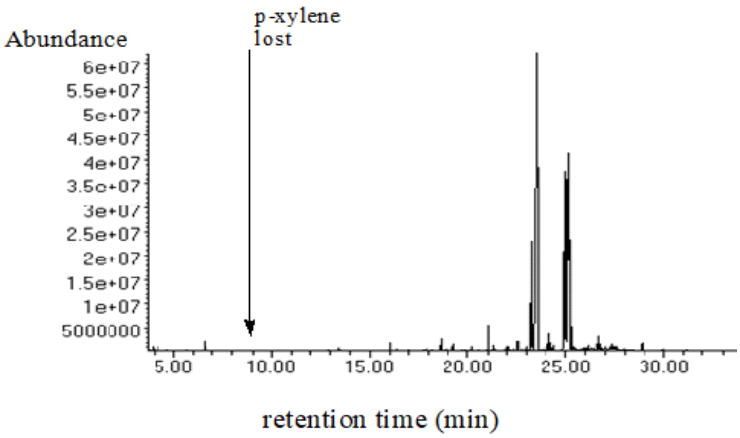

Figure 2: GC- MS profile for dried Spirulina soxhlet hexane extract (Absorbance vs time in minutes)

Heptadecane, derived from fatty acids, identified as major component in wet *S. platensis* and other hydrocarbons such as octadecane, pentadecane and hexadecane identified, are known to be the character impact compounds of edible algae and contributes to the odor and off-flavor in water.[18] The two hydrocarbons (pentadecane and hexadecane) disappear completely on drying the Spirulina, probably due to their low boiling point. On the other hand, phytol, earlier reported from this species in other parts as the major compound, [19] could be a characteristic compound influencing the flavor of S. platensis and contributes to fresh and floral odors.[20] Other aroma compounds, such as the aliphatic acids, including hexadecanoic acid, volatile alcohols and ketones also play a significant role in the production of foods and this improves its flavor.

Most of these compounds identified have been reported to have high biological activities.[21] Previous reports have shown compounds such as octadecane and heptadecane were found in both algae and plant species which showed potent antioxidant, anticancer and antimicrobial activity.[22, 23] In the present study, octadecane and heptadecane were identified in both wet and dried samples. Hexadecane, heptadecane, octadecane, phytol and pentadecane of hexane extract of wet Spirulina platensis identified by GC-MS have been previously been reported to have antibacterial activity against *Staphylococcus aureus* and *Salmonella typhimurium*.[24] GC-MS analysis of ethyl acetate extract of *Anabaena variabilis, Oscillatoriaanustissima* and *Anabaena flosaquae* revealed the presence of heptadecane, octadecane, hexadeconic acid. These compounds are known to have potential antimicrobial activity against gram positive, gram negative bacteria, yeast and fungi. [25]

Majorly, the above compounds identified have low toxicity for using in folk medicine, food, perfume, cosmetic and pharmaceutical industries, as defoaming agents and to improve shelf-life and safety of minimally processed fruits. [26]

4. Conclusion

The study revealed a variety of chemical composition with characteristic of fatty acids and esters extracted from *Spirulina* and analysis by GC-MS. The results obtained add to the knowledge on the chemical composition of algae belonging to the genus Spirulina and help provide further explanation for their striking smell on drying. The results confirm that some volatile compounds (e.g p-xylene) can easily be removed while others can be reduced to small amounts (e.g phenylethyl alcohol), by complete drying of the fresh harvested *Spirulina* species. These results helps characterize that species investigated indicate the presence of some biologically active compounds present and give the chemistry of the aroma produced by the fresh species and after drying. Spirulina species may be used as a flavor in foods and its safety enhanced by drying. Further research should be done to isolate and characterize natural products in both dried and wet samples as well as biological assays of these extracts.

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Supporting Information Available: A table showing the detailed GC-MS analysis of volatile compounds in hexane extract of *Spirulina platensis*. This material is available at https://kenyachemicalsociety.org/journals

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