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FLUORIDE CONTENT IN DIETARY SUPPLEMENTS OF SPIRULINA (*ARTHROSPIRA* SPP.) FROM CONVENTIONAL AND ORGANIC CULTIVATION

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ABSTRACT: Spirulina (*Arthrospira platensis*) is a microalga with hypolipidemic, hypoglycaemic, anticarcinogenic, and anti-inflammatory properties. It is a rich source of proteins and vitamins, as well as micro- and macro-nutrients, which makes it an increasingly popular dietary supplement. However, there is a risk that spirulina supplements may act as a source of fluorine in human nutrition. Significant intake of this element has negative effects on the human organism. The aim of the study was to determine the content of fluoride in 34 spirulina supplements in powder and tablet form, originating from conventional and organic farming. The fluoride content in the supplements included in the study ranged from 39.11 ± 17.9 to 73.71 ± 43.01 mg/kg. Fluoride content in supplements in tablet form was significantly higher (p=0.0241). No statistically significant differences in the fluoride content were observed depending on the method of cultivation (conventional vs. organic).

Keywords: *Arthrospira* spp.; Cultivation method; Fluoride; Spirulina; Supplement form.

INTRODUCTION

Spirulina is a biomass of microscopic cyanobacteria, that is blue-green microalga, found naturally in the lakes of Mexico and Africa.[1] It is an increasingly popular dietary supplement, because of its numerous health-promoting properties. The compounds found in spirulina act as antioxidants, regulate carbohydrate metabolism, stimulate the immune system, and exert anti-inflammatory, anticarcinogenic, and bactericidal effects.[2] Spirulina was also reported to exert positive effects on blood pressure,[2] carbohydrate metabolism,[3] and lipid profile with reductions in the blood cholesterol levels.[4,5] Observations on animal models additionally demonstrated that spirulina supplementation increased the activity of lipoprotein lipase[6] and insulin secretion in the pancreas.[7]

Spirulina has been used for centuries as a source of human and animal nutrition, and in the 1960s became popular as a mass product.[9] Research has shown that spirulina contains bioactive compounds with antioxidant, anti-inflammatory, and other properties, including polyphenolics[10] and pigments, such as phycocyanin and allophycocyanin.[11] It also contains large amounts of proteins, vitamins, and essential fatty acids, including linoleic acid and gamma-linolenic acid. It also provides a source of many minerals including fluorine.[12]

Fluorine is the most reactive element in the periodic table, which is found in the natural environment in the form of fluoride (F).[13] The main source of F in human nutrition comes from drinking water.[14] The other natural sources of F in the diet include grain products, leafy vegetables, nuts, potatoes, fish, and tea.[15] Excessive intake of F, or the chronic exposure even to its low concentrations, have negative effects on the organism. When administered orally in large doses, it causes acute

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intoxication (shallow breathing, spasms, salivation, and nausea). The regular intake of small doses of F leads to a chronic intoxication.[16,17] It has been proved that F has a toxic influence on the activity of specific inflammatory enzymes and free radical processes, as well as on several organs, such as kidneys, the liver, and the brain.[18-21] Considering this information, we decided to determine the content of F in commercially available spirulina supplements in tablet and powder form, originating from conventional and organic farming.

MATERIAL AND METHODS

Material: The material used in the study was spirulina, in tablet and powder form, marketed as a dietary supplement. A total of 34 spirulina samples, from different countries of origin, were obtained from specialist shops. The samples in tablet form were ground thoroughly using a mortar and pestle under aseptic conditions before the analysis.

Determination of F content: 0.01 g of the powder was weighed and placed into plastic tubes. Then, 1.0 mL of perchloric acid was added and incubated in a thermal cycler (95ºC, 1 hr). F concentrations in individual samples were measured by the potentiometric method with a fluoride ion-selective electrode (Orion 9409 BN, Thermo Scientific, USA). From the sample prepared in this way, 0.5 g was taken and mixed with 2.5 mL TISAB II and 2.0 ml of trisodium citrate. After mixing, the potential difference of each sample was measured for 10 min; 5 min before the addition of the appropriate standard, and 5 min after the addition. According to the work of Łukomska et al.,[22] the F content in the samples was calculated based on the difference of potentials measured in each sample and the concentration of the added standard. The electrode was calibrated using standard solutions. The correctness of the analytical procedure was controlled by comparing the concentration of F in the NaF solutions with NaF solutions of known concentrations (0.1, 1.0, and 10.0 mg/L).

Statistical analysis: In all the experiments, three samples were analysed and all the assays were carried out at least in triplicate. The statistical analysis was performed using Stat Soft Statistica 13.0 and Microsoft Excel 2010. The results are expressed as mean values and standard deviation (SD). The distribution of results for individual variables was obtained with the Shapiro–Wilk test. As most of the distributions deviated from the normal Gaussian distribution, non-parametric tests were used for further analyses. To assess the differences between the studied groups, the non-parametric U Mann–Whitney test was used.

RESULTS

The F content in the spirulina supplements subject to testing varied widely. The highest content of the analyzed element was observed in a conventionally grown spirulina supplement in tablet form. On the other hand, the lowest result was obtained in a spirulina supplement in tablet form from organic farming (Table). The differences in F content in the examined samples were also statistically analyzed including both the form of the supplement and the cultivation method (Table). Statistically significant differences in the fluoride content were observed between the spirulina from conventional cultivation in tablet and powdered form and the spirulina in tableted supplements from organic cultivation ($p=0.002$ and $p=0.024$, respectively, Table).

Table. Fluoride content [mg/L] in the spirulina samples, including form of the supplement and method of cultivation

Number of samples	Supplement form	Cultivation method	F content [mg/kg] Mean (±SD)
8	Powder	Conventional	39.11(±17.90) *b
16	Tablet	Conventional	73.71(±43.01) *a, c
10	Tablet	Organic	49.57(±28.55) *b

* $p<0.05$ between particular subgroup: a – powder/conventional, b – tablet/conventional, c – tablet/organic

The content of F in the spirulina samples was diversified depending on the form of supplement. A higher mean value was obtained for spirulina supplements in tablet form (64.43±39.33 mg/kg) than for those in powder form (39.10±17.90 mg/kg) (Figure 1). The observed difference was statistically significant (p=0.024).

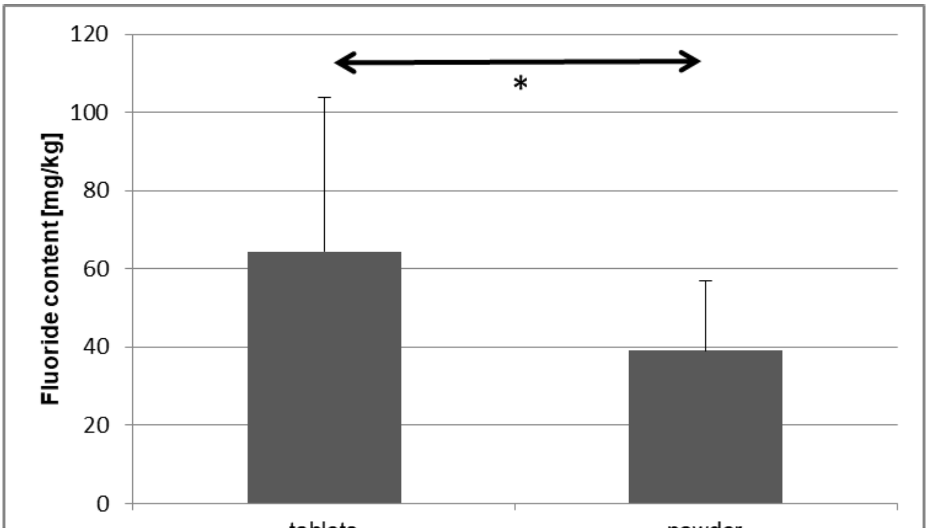

Figure 1. Fluoride content in spirulina supplements depending on the form of supplement.

The mean F content was also analyzed for differences depending on the spirulina cultivation method. The mean F content in conventionally grown spirulina samples was higher (62.18±39.78 mg/kg) than in organic spirulina samples (49.58±6.64 mg/kg) (Figure 2). However, these differences were not statistically significant.

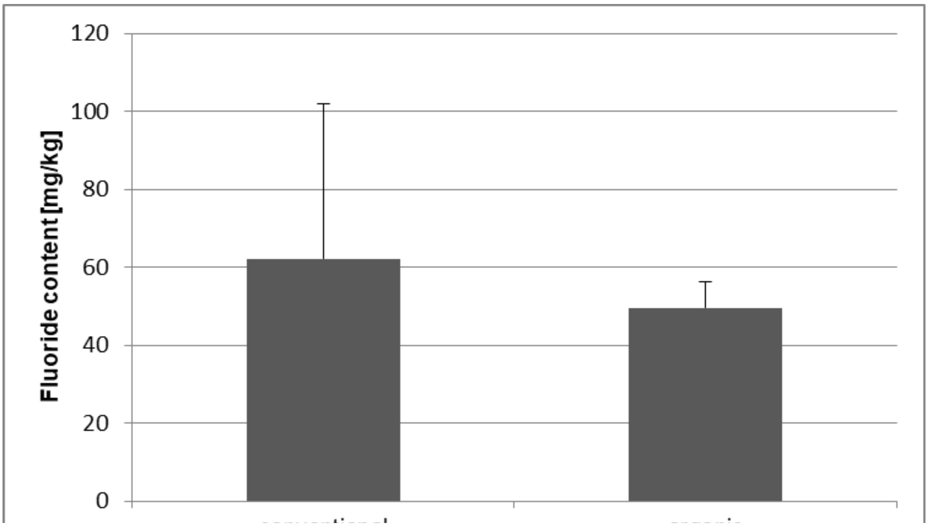

Figure 2. Fluoride content in spirulina supplements depending on the cultivation method

DISCUSSION

Cyanobacteria, including spirulina, have powerful accumulating properties. By secreting chelating agents, they can bind substances like metals or trace elements.[23] Spirulina has stronger binding properties than *Chlorella vulgaris,* which means that its consumption may be a source of elements toxic to humans.[24] Spirulina samples have been observed to contain heavy metals, such as lead, mercury, cadmium, and arsenic.[25] In one study on spirulina supplements, originating from different countries, it was reported that, in terms of heavy metals, nickel and zinc were found in the highest concentrations, and mercury and platinum were found in smaller quantities.[26] Despite the above, spirulina is an increasingly popular dietary supplement, because of its numerous health-promoting properties resulting exactly from its ability to bind a large amount of the trace elements necessary to maintain the homeostasis of the body. By secreting chelating agents, this microalgae can bind substances like metals or trace elements. However, strong accumulating properties of spirulina may lead to it being a significant source of toxic substances, including F.

F content in spirulina supplements can be influenced by many factors, such as the place of origin, the F content in the water,[27] environmental pollution, industrial development of the growing area[28] the cultivation method, and the form in which the spirulina supplements occur. Elevated F levels in the environment are caused by economic development.[29,30] Proximity of factories and a high level of industrialization[31] combined with increased usage of agricultural fertilizers, enriches the F content in soil, air, and groundwaters.[32]

Our study shows that taking spirulina in tablets is associated with a higher F exposure than the consumption of spirulina powder. This may be due in part to the manufacturing process which involves wet blending of ingredients with the use of water.[33] Such processing may increase the F content in the finished product, if significant F levels are present in the water used.

While there have been no studies to date on the F content in dietary supplements, there are reports on the F content in groundwater. The optimal growing conditions for spirulina are found in tropical and subtropical waters, which tend to have an alkaline pH.[34] It is found, that alkaline waters contain higher amounts of bioavailable F.[32] Commercially, spirulina is produced in large outdoor ponds under controlled conditions.[35] Rarely is it being sourced directly from lakes.[36] Due to the accumulating properties of spirulina, the F levels in the tested samples may depend on its levels in the water used as the cultivation medium for that microalga. Water F levels differ depending on geographical location, as evidenced by studies conducted in different regions of the world. Water F content determined by Prasad et al. in Fiji ranged from 0.02 to 0.19 mg/L.[37] The average concentration of F in the waters of Tibet amounted to 0.06 mg/L.[38] An even higher F content, reaching up to 2.15 mg/L, was observed in the groundwaters of Korea.[39]

The place of origin of the analysed samples may also have an affect on the content of F. The spirulina samples included in the present analysis originated from the countries with a high degree of economic development, such as European countries, China, and the USA. However, the exact growing locations were not provided by the suppliers. Additionally, it needs to be highlighted that in 11 out of the 34 supplements analysed the supplier did not provide the country of origin for the material at all.

Suppliers promise that organic cultivation relies on water from groundwater sources and ocean water. However, the F content of those waters is undetermined. Our analysis showed that spirulina grown through organic cultivation had a lower level of F contamination than conventionally grown spirulina. However, the difference was not statistically significant. The scope of study should therefore be broadened to account for the area of origin of spirulina, with a simultaneous analysis of environmental F levels. Water fluoridation in some countries should also be considered as a contributing factor with regard to increased F levels in spirulina supplements.

Our study demonstrates that spirulina supplements are a source of F. It remains to be considered, however, whether F concentrations are sufficiently high to render spirulina supplementation a risk of excessive F exposure. Depending on the supplier, the recommended daily intake of spirulina was listed as ranging from 750 mg to 10 g. The recommended dietary allowance (RDA) for F is 3 mg/day.[40] Looking at the mean content of the analyzed element in the supplements in powder form, it may be concluded that the daily intake of F may range between 0.032 and 0.429 mg per day, and in the case of the supplements in tablet form, the daily intake will range from 0.047 to 0.630 mg F^-. Therefore, daily supplementation with spirulina may provide up to 21% of the RDA for F.

It must be remembered that plant products, such as vegetables[41] and fruits[42] as well as herbs and beverages,[43,44] provide additional F sources in human nutrition. The largest amounts of F, however, come from drinking water, including tap water[45] and bottled waters.[46] When introducing spirulina supplementation, one must be aware of the risk of exceeding the permissible daily limits for F exposure.

CONCLUSIONS

In the light of the analysis above, it can be concluded that spirulina supplements are a substantial source of F in human nutrition. The study demonstrated that spirulina cultivation method did not have a significant effect on F concentrations, and supplements originating from organic cultivation did not have significantly lower F levels. On the other hand, a significant difference was observed in the F content of supplements sold in tablet vs. powder form.

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