Screen for cyanotoxins in cyanobacteria based food supplements on the Belgian market

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Contaminated supplements

Microcystin congeners found in food supplements. Sample *Apha*-430 contained a total concentration above the suggested EFSA limit (1ng/g)[1,7]. All the contaminated *Apha* samples originated from the Klamath Lakes. A toxin-producing genera like *Microcystis* might be co-occurring in the harvested bloom, provided that *Apha* does not produce MCs.

Cyanobacteria, growing in lakes and ponds, have been used as a food source for centuries. Important examples are *Arthrospira sp.* from lakes in South America and Ouadi in Africa, also known as 'spirulina'. More recently, *Aphanizomenon flos-aquae (Apha)* is being harvested from blooms in the Klamath Lake in Oregon. Besides natural harvest, *Arthrospira sp.* and (the micro-algae) *Chlorella vulgaris* are being produced in closed cultivation tanks or open artificial ponds.

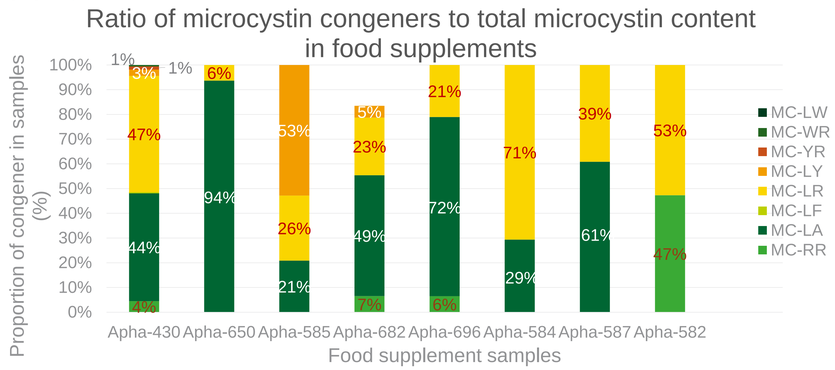
However, several studies have shown that some of these finished products can be contaminated with toxins of cyanobacterial origins. Mainly, microcystin congeners are found. These hepatotoxins are produced by a number of cyanobacterial species, generally from the genus *Microcystis.* The mechanism of contamination is still unclear[1-5].

We tested 35 different food supplements using a previously validated LC-MS/MS method to quantify eight microcystin congeners (MCs) and nodularin (NOD)[6].

Methods

- During market, 40 stores were visited and a web search was performed
- Tablets were crushed with a mortar.
- Capsules were opened and the powder was collected.
- Extraction with 80% ethanol in an overhead shaker.
- Separation on a Waters BEH C18 column (1,7 µm, 2,1x100mm)
- Flow rate of 0,5 ml/min.
- Gradient from 98% aqueous to 98% organic phase was used. Each phase was supplemented with of 0,25% formic acid
- Toxin detection on a Waters Xevo TQ-S
- Quantifier and qualifier for each compound (Table1).

Fig. 1: Proportion of individual congeners in food supplements compared to total MC content displayed for each sample. Congeners with a prevalence below 1% are not shown.



Results

- Out of 35 samples, 8 contained microcystin congeners > Limit of Quantification (18 ng/g).
- Common congeners were MC-LA and MC-LR (fig. 1)
- Half of the samples also contain MC-RR (fig. 1)
- MC-LY was present in three samples (fig. 1)
- NOD was not detected in the samples
- Sample *Apha*-430 contained the highest concentration of MCs, 4,837 ng/g. In this sample, low concentrations of MC-LF, MC-WR and MC-YR could also be found (Table 2).

Table 1: Mass transitions for the microcystin congeners and nodularin

Cyanotoxin	Precursor ion (m/z)	Quantifier ion (m/z)	Qualifier ion (m/z)
MC-LR	995.4	135.0	213.1
MC-RR	519.8	134.8	107.2
MC-YR	1045.5	135.3	212.9
MC-WR	1068.4	135.3	213.1
MC-LY	1002.4	135.3	213.0
MC-LA	910.3	135.1	107.1
MC-LF	986.3	135.0	213.1
MC-LW	1025.4	134.9	213.1
NOD	825.25	134.9	102.7

Table 2: Results for detected microcystin congeners in cyanobacteria based food supplements. In total, 35 different food supplements were tested of which 8 contained MCs.

Concentration (ng/g) ► Samples ▼	MC-RR	MC-LA	MC-LF	MC-LR	MC-LY	MC-YR	MC-WR	MC-LW	Total MC-LR equivalent
***Apha*-430**	217,5	2111,8	18,4	2276,5	125,5	62,3	25,3	<LOQ	**4837,4**
***Apha*-582**	18,5	<LOQ	<LOQ	20,6	<LOQ	<LOQ	<LOQ	<LOQ	**39,1**
***Apha*-584**	<LOQ	91,8	<LOQ	220,6	<LOQ	<LOQ	<LOQ	<LOQ	**312,4**
***Apha*-585**	<LOQ	123,95	<LOQ	156,3	313,8	<LOQ	<LOQ	<LOQ	**594,1**
***Apha*-587**	<LOQ	94,9	<LOQ	61	<LOQ	<LOQ	<LOQ	<LOQ	**155,9**
***Apha*-650**	<LOQ	713,8	<LOQ	48	<LOQ	<LOQ	<LOQ	<LOQ	**761,8**
***Apha*-696**	35	392,2	<LOQ	113,8	<LOQ	<LOQ	<LOQ	<LOQ	**541**
***Apha*-682**	35,7	267,7	<LOQ	127,5	26,2	<LOQ	<LOQ	<LOQ	**547,4**

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