



Letter to the Editor

RE: Protein bioavailability of *Wolffia globosa* duckweed, a novel aquatic plant, a randomized controlled trial



We are thankful for the justified questions of Dr. Christine Dawczynski and her colleagues regarding the presence of an active B12 vitamin in this specific plant, as this is, indeed, a unique phenomenon, which deserves further studies. Thank you for the opportunity to clarify and to add new accumulated information.

The reported vitamin B12 levels in the *Wolffia globosa* duckweed (Mankai strain) are based on the analysis performed by Eurofins labs and were determined by the microbiological assay - AOAC952.20 (modified). This method, based on *Lactobacillus delbrueckii* subsp. *Lactis*- ATCC 7830, is the official AOAC method for detection of vitamin B12 in food for many years.

As mentioned in the letter, it is known that plants contain a large spectrum of corrinoids and an analytical differentiation between active and inactive corrinoids is required before declaring on active vitamin B12 content. As concluded by Watanabe and Bito [1] the B12 bioassay method is not sufficient to ensure active B12 presence and identification methods such as TLC, HPLC, or LC/MS-MS are suggested to verify the active vitamin B12 form. Here we firstly report a study performed in collaboration with the Weizmann Institute, Life Sciences Core Facilities Laboratory, to validate the vitamin B12 presence in Mankai by using the UPLC/MS-MS method. Various plant samples from different growth conditions were analyzed by Liquid Chromatography (Acquity I-class UPLC system) and MS (Xevo TQ-S triple quadrupole mass spectrometer) following KCN-boiling extraction method [2,3]. The results revealed that in all samples the B12 form has the same retention time and mass as the standard cyanocobalamin vitamin B12 used (Sigma V2876). Those results clearly showed that Mankai contains active vitamin B12.

It is well known that B12 is synthesized by certain bacteria and archaeon, but not by plants or animals. Therefore, the involvement of endophytic bacteria in the synthesis of B12 in Mankai was suggested by us. Plant endophytic bacteria is a well-known natural phenomenon including all crop plants. Usually, plant endophyte bacteria do not pose any pathogenesis risk for humans. Nevertheless, Mankai products successfully passed the OECD toxicological studies, including genotoxicity and mutagenesis, with no evidence for any toxicological effect.

The electrochemiluminescent immunoassay "ECLIA" method that was used in the clinical studies, and which is based on the

human intrinsic factor (hIF), is a common method to measure vitamin B12 in blood. Indeed, this assay cannot completely differentiate cobalamins from inactive corrinoids. However, the affinity of the vast majority of the corrinoids to hIF is significantly lower. It was reported by Stupperich and Nexø [4] that the affinity of pseudo B12 to hIF is 500 fold lower than cobalamin.

The primary aim of this study was to compare the bioavailability of the essential amino acids of this specific *Wolffia globosa* duckweed to equivalent well known plant and dairy protein sources. For the same amount of protein we had different levels of B12, but yet, Mankai, apparently, is happen to be an alternative source of cheese like protein and probably to induce by its own endophytes, that are being identified now days, the active B12 required for humans. However, as suggested by the authors, further research in humans is of urgent need to draw further conclusions and we welcome any collaborations.

References

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