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Directorate-General
Animals, Plants and Nutrition
Department
Foodstuffs, Animal Feed
and Other Consumer Products

Advisory Commission for Plant Preparations

Advice of 16th of December 2021 emitted by the Advisory Commission for Plant Preparations concerning the determination and labelling of Vitamin B12 content in plant products

The Advisory Commission for Plant Preparations was asked by the Directorate-General Animals, Plants and Food of the Federal Public Service Health, Food Chain Safety and Environment to issue an advice on the determination and labelling of vitamin B12 content in plant products;

Regarding the Royal Decree of 31 August 2021 concerning the manufacture of and trade in foodstuffs composed of or containing plants or plant preparations, and in particular Article 5, §6;

Considering the following information:

Vitamin B12 (Fig. 1) is only produced by certain bacteria. These vitamin B12-producing microorganisms are the biological source of vitamin B12. Animals and plants do not synthesise this vitamin, but in e.g. ruminants, vitamin B12 will be produced in the rumen stomach by the bacteria present, making the meat of these animals a good food source. Therefore, the natural food sources of vitamin B12 are animal foods such as meat, liver, milk, eggs, fish and shellfish.
Strict veganism thus poses a risk of vitamin B12 deficiency (Russcher et al., 2011; Rizzo et al., 2016), although it has been reported that plants living in symbiosis with certain bacteria or fermented plant foods could be sources of vitamin B12 (Rizzo et al., 2016; Nakos et al., 2017)

Some seaweeds (e.g. *Porphyra umbilicalis*), certain mushrooms (e.g. *Cantharellus cibarius*), and fermented foods (e.g. sauerkraut, fermented soy products such as natto, tempeh) could be a source of vitamin B12, but the data do not yet adequately demonstrate that it is effectively the active form of vitamin B12. For example, spirulina was shown to contain a concentration of 127-244 µg/100 g (dry weight), but 80 % of this was found to be the **inactive pseudo-vitamin B12** (Fig. 2) (Watanabe et al., 1999).

Fermented vegetables, such as sauerkraut, natto and tempeh, could also contain significant quantities of vitamin B12. But the presence of vitamin B12 in these foods depends on the bacteria present during the fermentation process and it is very difficult to standardise the amount of vitamin B12 in these fermented products because there **is variation in the microbiota during the fermentation processes**. For tempeh, for example, it was demonstrated that the amount of cobalamin formed during soybean fermentation can vary in the range of 0.7 to 8 µg per 100 g (Nout and Rombouts, 1990; Denter and Bisping, 1994).

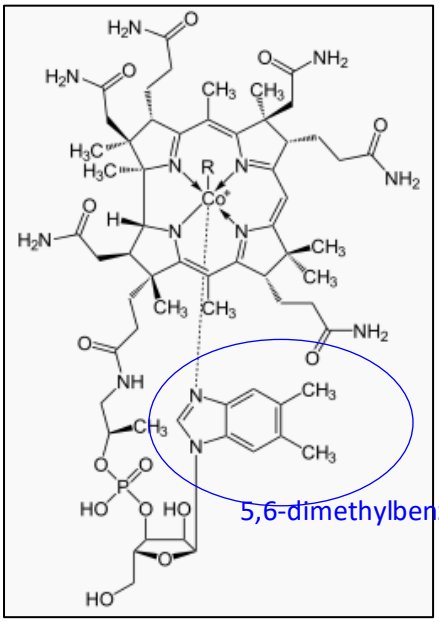

Fig. 1: Vitamin B12 (R= -CN: Cyanocobalamin; R=-OH: Hydroxocobalamin; R=-CH_3: Methyl-cobalamin; R= 5'-deoxyadenosyl: Adenosyl-cobalamin)

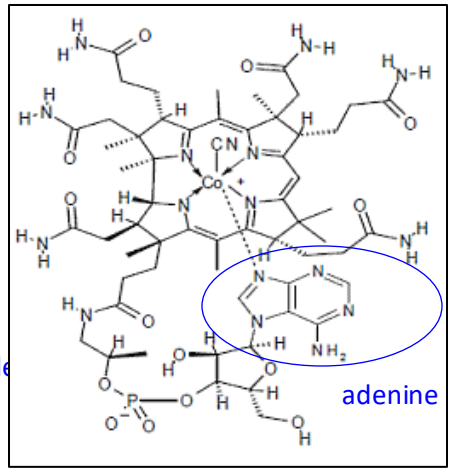

Fig. 2: Pseudo-vitamin B12

Older microbiological **methods of analysis** are inadequate for determining vitamin B12 in plants, as they measure both active B12 and inactive analogues and are unable to distinguish between the different forms (Rizzo et al., 2016). Classical HPLC-UV is also not sensitive enough to measure vitamin B12 in plants, due to its very low concentration and matrix effects (Nakos et al., 2017).

Recently, analytical methods have been published that do allow for a distinction to be drawn between active vitamin B12 and inactive pseudo-vitamin B12. For accurate determination of vitamin B12 in plants or plant foods, a **purification and concentration step using an immuno-affinity column** is necessary in sample preparation (Nakos et al., 2017; Watanabe et al., 2012).

The 5,6-dimethylbenzimidazole structure of active vitamin B12 (Fig. 1) is essential for binding to intrinsic factor in the gut and absorption of vitamin B12. Inactive vitamin B12 analogues have a structural difference, e.g. adenine instead of 5,6-dimethylbenzimidazole in pseudo-vitamin B12 (Fig. 2). In order to draw this distinction, and thus to be able to analyse vitamin B12 alongside its inactive analogues, **HPLC-(ESI)-MS/MS** analysis is required (Nakos et al., 2017; Tanioka et al., 2014; Gentili et al., 2008).

In conclusion, we can state that

- Depending on the analytical method used, inactive analogues of cobalamine in plant-based foods may be improperly detected as active cobalamine. Based on current information, the possibility that products contain an inactive analogue of vitamin B12 cannot be excluded. Operators need to be aware of this

- On the other hand, it appears that the content of vitamin B12 in certain plant products (e.g. fermented products) can vary greatly, since production depends on the fermentation process and the composition of the microbiota, which can be variable

The Advisory Commission on Plant Preparations concludes that

- In order to determine and report on the effective content of vitamin B12 in plant products, the use of a selective analytical method for the active form of vitamin B12 is recommended (HPLC MS/MS).
- As a result of the variability during fermentation processes, the content of vitamin B12 in fermented products also appears to be highly variable. Analysis of each lot of these foods is recommended if vitamin B12 levels are to be reported;
- Only the content of the active form of vitamin B12 may be listed on the label.

The Advisory Commission on Plant Preparations reserves the right to re-examine this advice in the light of new considerations.

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