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Some Plant Food Products Present on the Polish Market Are a Source of Vitamin B12

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Abstract: Cyanocobalamin is the most widespread form of vitamin B12, which is sufficient for humans. Vitamin B12 is mainly found in animal products. However, supplementation does not have to be necessary because certain amounts of vitamin B12 are present in plant products. Previous studies showed significant contents of cyanocobalamin in sea buckthorn and in sauerkraut. In this study, selected products such as sea-buckthorn jam and fermented plant products (obtained by lactic acid fermentation) were tested in a search for vitamin B12. Bacteria involved in this type of fermentation have the potential to produce cyanocobalamin. Popular fermented plant products on the Polish market were selected, namely sauerkraut and pickled cucumbers, as well as parsley juice, beetroot juice and white borscht. The analysis was carried out using HPLC-UV. Most of the analyzed products did not contain significant levels of vitamin B12. Only sea-buckthorn jam and pickled parsley juice can provide the amount of vitamin B12 needed to prevent deficiency.

Keywords: vitamin B12; cyanocobalamin; cobalamin; fermented plant products; HPLC-UV; vegan diet

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1. Introduction

Vitamin B12, also called cobalamin, is the most complex of all vitamins [1]. It is a key cofactor for the methylation process, essential in reactions related to DNA and in the metabolism of cells [2], as well as a key ingredient in the process of erythropoiesis. Therefore, its insufficient intake can cause negative effects on the homeostasis of the human body [3]. Mild deficiency is manifested by fatigue and anemia. Moderate deficiency is often associated with the occurrence of macrocytic anemia and the initial stage of neurological problems manifesting, among others, polyneuropathy. With a significant deficiency, the suppression of bone marrow function occurs along with serious neurological problems and an increased risk of cardiomyopathy [4,5]. Deficiency of vitamin B12 also increases the risk of DNA damage [6].

Human requirements for vitamin B12 vary and depend, among others, on age and physiological condition. According to data from the European Food Safety Authority (EFSA), a daily intake of 1.5–2 µg cobalamin is the minimum requirement for maintaining a healthy hematological condition. Based on various factors and on the estimation of daily cobalamin losses, it was suggested that the daily requirement of cobalamin ranges from 4 to 20 µg. Therefore, an adult should intake 4.0 µg of cobalamin per day with food. The demand for this vitamin increases in pregnant women (4.5 µg of cobalamin per day) and lactating women (5.0 µg of cobalamin per day) [7].

People consume vitamin B12 from food. It is present in negligible amounts, if at all, in some plants, including vegetables, fruits and algae [1,8–14], but mostly occurs as a result of the activity of microbes inhabiting the digestive tract of mammals, mainly ruminants. Therefore, meat and animal products are considered the best source of vitamin B12 in the human diet [15,16].

People on a restrictive vegetarian diet, in particular vegans, are most at risk of vitamin B12 deficiency. The complete exclusion of the richest source of cobalamin, i.e., animal products, may be associated with negative health consequences. Conscious vegans support their diet by supplementation or by pharmaceutical preparations. In order to avoid vitamin B12 deficiency, some food producers fortify their products. However, many vegetarians tend to avoid processed foods [17].

Because vitamin B12 can be produced by bacteria found in the digestive tract of animals, it should also be synthesized during the lactic acid fermentation process. Lactic acid bacteria (LAB) are naturally present in the human digestive system and manifest the ability to synthesize B vitamins [18,19]. Recently, selected bacteria were shown to be able to synthesize cobalamin. In 2003, there were indications that bacteria from the *Lactobacillus* genus, in particular *Lactobacillus reuteri*, could effectively produce vitamin B12 [20]. An even greater amount of vitamin B12 was obtained by the controlled fermentation of plant products using a combination of LAB and propionibacteria [21]. In 2007 Santos et al. showed that *Lactobacillus reuteri* is able to produce pseudovitamin B12 under beta-oxygen conditions. Pseudovitamin B12 differs from cobalamin in the α-ligand where it has adenine in place of the 5,6-dimethylbenzimidazole α-linked with the C-1 ribose [22].

Lactic fermentation is a natural food preservation method that has been applied for a long time. It is used to preserve and extend the shelf life and improve the taste, texture and functional properties of foods [23]. Both animal and vegetable products can be fermented. In Poland, the most popular fermented vegetables are white cabbage (sauerkraut) and cucumbers. Besides these plant materials, red cabbage, red beets, sorrel, tomatoes, peppers, carrots and many other vegetables can be also used. Fermented vegetable, fruit and vegetable-fruit juices are also quite popular in Poland. They are produced by the lactic fermentation of juices obtained from vegetables and fruits. Fermented cereal products are also worth mentioning. Among them, white borscht is a liquid substance that is produced by the fermentation of flour, water and spices. The product, available in stores, is used to prepare a traditional Polish soup of the same name. Sour rye soup has a very similar use and taste; the only difference is that wholemeal rye flour is used instead of wheat flour for its preparation. Ready sourdough is used to prepare traditional Polish soups such as żurek and white borscht. A combination of yeasts and LAB from rye bread or cracked rye, wheat, barley and millet, with the addition of flour, produces a refreshing, nonalcoholic beverage named kvass. This drink effectively quenches thirst, stimulating appetite and digestion [24].

The works by Babuchowski [21] and Nakos et al. [12] were the inspiration behind starting research in this area. Both studies were conducted using various plant products. The first study showed that vitamin B12 was present in plant products subjected to controlled fermentation. On the other hand, Nakos et al. [12] studied single species of mushrooms and plants such as garlic, couch grass, parsnip, corn poppy and sea buckthorn along with related products, in which significant contents of vitamin B12 were determined. These reports prompted us to conduct a similar study, analyzing sea-buckthorn jam, sauerkraut and other items. One novelty in this work is the extended range of fermented products, which, according to available knowledge, had not been analyzed before. Moreover, the studied products were commercial and not prepared in a controlled manner.

The aim of this study was to estimate the content of vitamin B12 in selected popular fermented plant products and confirm the significant content of this vitamin in the sea-buckthorn products available on the Polish market, using high-performance liquid chromatography with ultraviolet detection (HPLC-UV).

2. Materials and Methods

2.1. Fermented Products and Sea-Buckthorn Jam

Fermented plant products and sea-buckthorn (*Hippophae rhamnoides* L.) jam traditionally consumed by Poles and widely available in stores in Poland were analyzed. The products were produced by various manufacturers. Samples of one jam, five different sauerkrauts, five different pickled cucumbers and the juices of these products were analyzed. Samples were also taken from two different bottled sauerkraut juices, one pickled cucumber juice, two pickled beetroot juices and three different white borschts, one sour soup and pickled parsley juice. The test samples were purchased from local stores in Lublin (Poland) and were immediately stored in a refrigerator for a maximum of three days until analyzed.

2.2. Reagents

Cyanocobalamin (vitamin B12) and methanol (gradient grade for HPLC) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (gradient grade for HPLC) was obtained from POCH (Gliwice, Poland).

2.3. Sample Preparation

Samples were prepared for HPLC analysis as described in detail in the method of Markopoulou et al. [25]. In particular, liquid samples were filtered using a syringe filter (pore size = 0.4 µm) directly prior to injection into the HPLC system. Solid samples were weighed (2 ± 0.001 g), thoroughly ground in a mortar and quantitatively transferred to falcons using 10 mL of distilled water. Directly prior to the injection into the HPLC system, samples were filtered through a syringe filter (pore size = 0.4 µm). Cyanocobalamin standard (340 µg) was freshly dissolved in 1 mL of water (stock solution), followed by preparation of a series of 10 dilutions. Each product was analyzed in duplicate.

2.4. HPLC

The methodology of HPLC analysis was based on the method described in Nakos et al. [12], with slight differences (without any significance for the obtained results).

Samples were analyzed in an HPLC system consisting of two Smartline 100 pumps, a dynamic mixer, a 20 µL loop, an Azura UVD 2.1S detector (360 nm) and an IF2 interface (all from Knauer, Berlin, Germany). A LiChrospher 100 RP18 C18 column (250 mm × 4.0 mm, particle size = 5 µm; Macherey-Nagel, Düren, Germany) coupled to a dedicated precolumn was used for separations. The gradient was formed by 0.6% HPLC-grade formic acid in distilled deionized (DDI) water (A) and HPLC-grade 80% acetonitrile in DDI water (B) with the progression of 0–5 min 0% B, 5–40 min 0–40% B, 40–43 min 40–80% B, 43–46 min 100–0% B and 46–50 min 0% B. The flow was 1 mL·min^{-1}, and the separation took place at laboratory temperature. The response signal was analyzed using Eurochrom 3.05 P5 software (2000 Basic Edition, Knauer). The calibration curve was constructed using 10 concentrations of vitamin B12 (0.1–50 µg·kg^{-1}), and the concentrations in the samples were calculated based on integration of the chromatographic peak with a retention time of 21 min. The absorbance wavelength was 361 nm. The limit of detection (LOD) value that was experimentally determined under the same conditions was 1.234 µg·kg^{-1}. The limit of quantitation (LOQ) value was 3.746 µg·kg^{-1}.

3. Results and Discussion

The range of linearity of the calibration curve with a correlation coefficient R^2 = 0.999 for vitamin B12 is shown in Figure 1.

Table 1 presents the content of cyanocobalamin (vitamin B12) in the studied solid samples. In the sea-buckthorn jam sample, a significant amount of cyanocobalamin was detected, up to 224.5 µg·kg^{-1} (Figure 2).

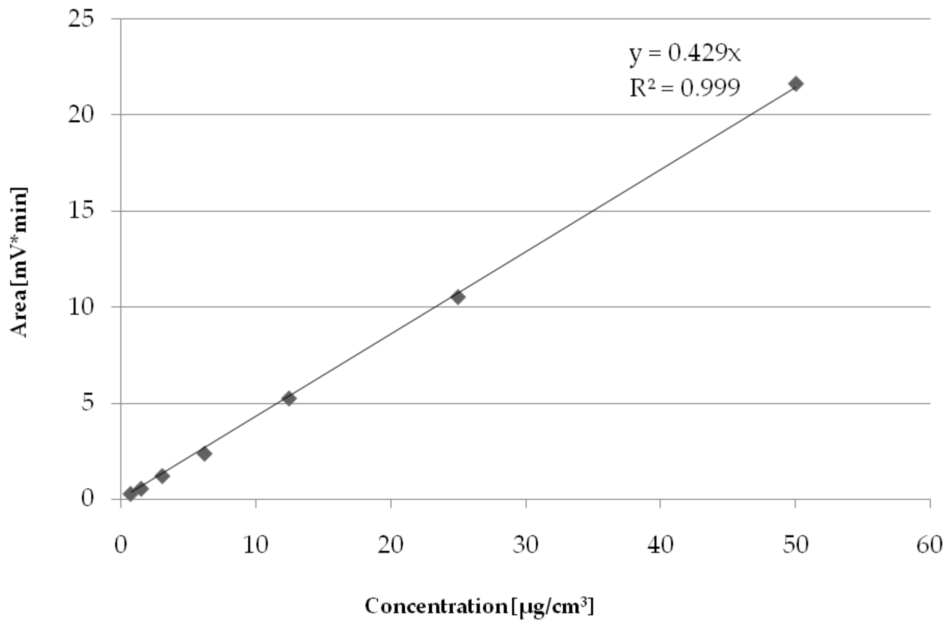

Figure 1. Calibration curve of cyanocobalamin.

Table 1. Content of cyanocobalamin in solid samples of fermented vegetable products.

Product	Manufacturer	Content of Vitamin B12 ($\mu g \cdot kg^{-1}$) ($\bar{x} \pm SD$)
Sea-buckthorn jam 100%	Łowicz	224.5 ± 0.5
Sauerkraut	Matyjaszczyk	Traces
Sauerkraut	Brassica	Traces
Sauerkraut	Dominik	Traces
Sauerkraut	Stefanek	Traces
Sauerkraut	Farma Świętokrzyska	Traces
Pickled cucumber	Matyjaszczyk	Traces
Pickled cucumber	Kuchnia Polska	Traces
Pickled cucumber	Stefanek	Traces
Pickled cucumber	Farma Świętokrzyska	Traces
Pickled cucumber	Raj	Traces

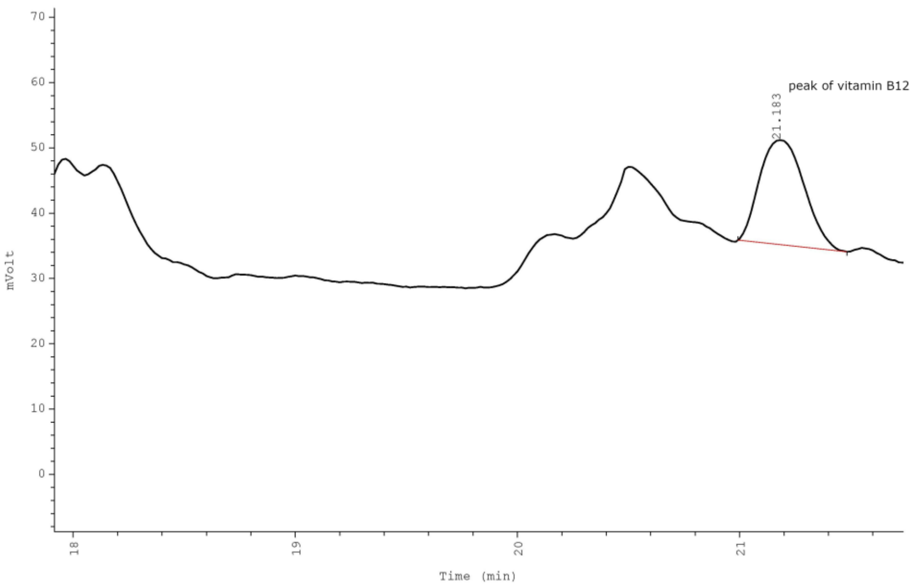

Figure 2. HPLC chromatogram obtained after the analysis of the sea-buckthorn jam sample.

In solid samples of sauerkraut of all manufacturers, only trace amounts of vitamin B12 were detected. The same results were obtained by the analysis of cucumber solid samples. In the next stage of the study, liquid samples of pickled cucumber and beetroot brines, as well as sauerkraut and parsley juices, were analyzed. Only the latter samples were found to contain significant amounts of vitamin B12, up to 50.5 $\mu g \cdot L^{-1}$, while other samples were found to contain only trace amounts of vitamin B12 (Table 2, Figure 3).

Table 2. Content of cyanocobalamin in liquid samples of fermented vegetable products.

Product	Manufacturer	Content of Vitamin B12 ($\mu g \cdot L^{-1}$) ($\bar{x} \pm$ SD)
Pickled parsley juice	BIOFOOD	50.5 ± 0.2
Sauerkraut juice	Matyjaszczyk	Traces
Sauerkraut juice	Brassica	Traces
Sauerkraut juice	Dominik	Traces
Sauerkraut juice	Stefanek	Traces
Sauerkraut juice	Farma Świętokrzyska	Traces
Sauerkraut juice	Kacuś	Traces
Sauerkraut juice	BIO FOOD	Traces
Pickled cucumber juice	Matyjaszczyk	Traces
Pickled cucumber juice	Kuchnia Polska	Traces
Pickled cucumber juice	Stefanek	Traces
Pickled cucumber juice	Farma Świętokrzyska	Traces
Pickled cucumber juice	BIO FOOD	Traces
Pickled cucumber juice	BIO FOOD	Traces
Pickled cucumber juice	Kuchnia Polska	Traces
Pickled cucumber juice	BIO FOOD	Traces
Pickled beetroot juice	BIOFOOD	Traces
Pickled beetroot juice	Kuchnia Polska	Traces
White borscht	Kuchnia Polska	Traces
White borscht	Tesco	Traces
Żurek	BIES	Traces

In other sauerkraut juices and fermented cucumber juices, only trace amounts of vitamin B12 were detected. Additionally, in samples of white borscht from three different manufacturers, only traces of vitamin B12 were detected.

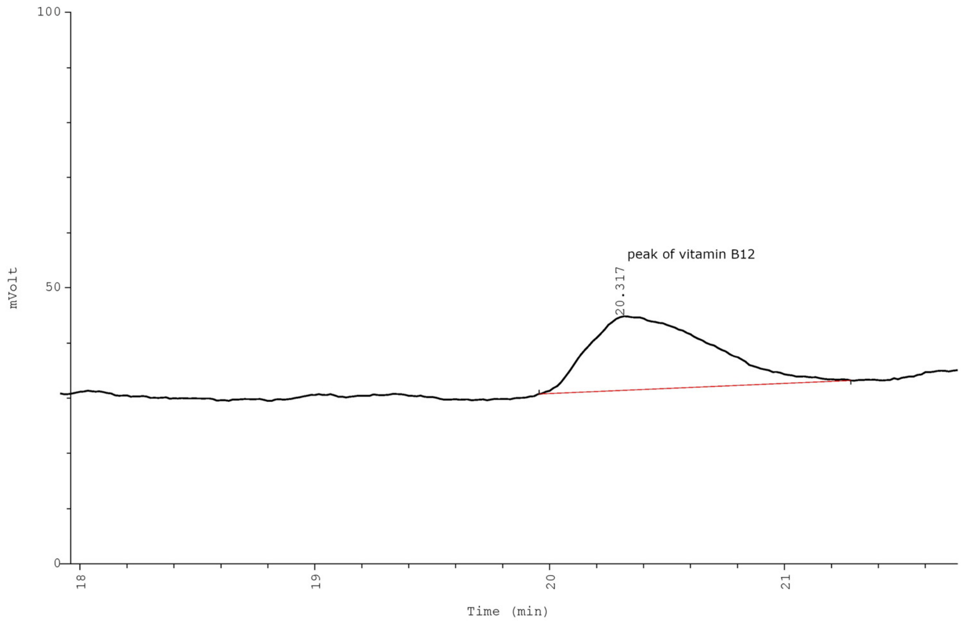

Figure 3. HPLC chromatogram obtained after the analysis of the pickled parsley juice sample.

This study used HPLC analysis to determine the content of cyanocobalamin in selected fermented plant products and sea-buckthorn jam available on the Polish market. The main intention of this study was to find a healthy, natural and easily available source of vitamin B12 for people at risk of deficiency, in particular vegetarians and vegans.

The scientific community is still looking for new and better vegetable sources of cobalamin. Mozafar [8] demonstrated that spinach grown with organic fertilizers (addition of cow dung at the rate of 10 $g \cdot kg^{-1}$) increased the B12 content in spinach leaves by close to two-fold (from 6.9 to 17.8 $ng \cdot g^{-1}$ dry weight). Some studies report that some mushrooms and nori seaweed contain trace amounts of vitamin B12 [9,10]. Vitamin B12 was also found in dried shiitake mushrooms (Lentinula edodes) with a content of approximately 56.1 $\mu g \cdot kg^{-1}$ of dry matter. Unfortunately, by a typical portion of spinach, mushrooms or nori, a consumer is not able to cover the daily requirement for cobalamin, and daily consumption of shiitake mushrooms at 50 g per day would even not be possible [1].

For many years, edible bamboo shoots were thought to contain significant amounts of vitamin B12, which ultimately proved to be a misconception [12]. Only trace amounts were found in broccoli, asparagus, Japanese butterbur and Mung Bean sprouts [10]. Still, other studies have found that edible species of black trumpet and golden chanterelle contain vitamin B12 at a 10.9–26.5 $\mu g \cdot kg^{-1}$ level of dry weight [13]. A good source of vitamin B12 may be the Mankai, Aphanizomenon and Nostocidible species of cyanobacteria, which are usually available as supplements [11,26]. Many fermented plant products were also analyzed in the past, including a Japanese type of fermented black tea (Batabata-cha), containing a significant amount of vitamin B12, up to over 4500 $\mu g \cdot kg^{-1}$ of dry tea leaves and 20 $\mu g \cdot L^{-1}$ of tea drink [14]. Unfortunately, such products are not popular in Central Europe, especially in Poland. Therefore, in our study, we examined products that are constantly in the diet of Poles.

In this study, the concentration of vitamin B12 in pickled cucumbers, sauerkraut, juices and other fermented products, as well as sea-buckthorn jam available on the Polish market were analyzed. All samples were obtained from ready-made products available from shops. Most samples were found to contain minor traces or no vitamin B12. Gupta et al. [27] reported more than half the amount of vitamin B12, in comparison with our results, in fenugreek leaf juice (120 $\mu g \cdot L^{-1}$). In the cited work, LAB also participated in the fermentation process. Babuchowski et al. [21], who analyzed sauerkraut, reported significantly different results. In their study, they used specific bacterial strains for fermentation and checked how much vitamin B12 they could produce. The fermentation process was carried out with LAB and a combination of LAB and propionicobacteria. In their study, the content of vitamin B12 reached 72 and 20 $\mu g \cdot kg^{-1}$ with and without the addition of propionicobacteria, respectively. The authors pointed out that such large amounts of vitamin B12 resulted from the fact that production was controlled compared to commercially made sauerkraut, which contained significantly smaller amounts of this vitamin (1.3 $\mu g \cdot kg^{-1}$). In this study, we found only traces of vitamin B12 in sauerkraut. Several factors, including differences in the microbiological composition of the products, storage conditions, age of the sauerkraut and the method of production, could have influenced this result. This may be a plausible explanation for why vitamin B12 could not be detected in our studies. A high content of vitamin B12 (50 $\mu g \cdot kg^{-1}$) was determined in pickled parsley juice. This would mean that half a glass of such juice would cover the daily requirement of an adult for vitamin B12.

In addition to fermented products, sea-buckthorn jam was also analyzed. A significant amount of vitamin B12 was determined (220 $\mu g \cdot kg^{-1}$). This would mean that an adult would only need to eat 18 g a day of this food product, which is about 1.5 tablespoons, to cover the daily requirement for vitamin B12. Similar results were obtained by Nakos et al. [12] by the analysis of sea-buckthorn fruits for vitamin B12. The authors determined that 100 g of dry matter contained as much as 37.01 μg of vitamin B12. This means that sea buckthorn may be the richest source of vitamin B12 among all plants.

4. Conclusions

By the HPLC-UV analysis conducted, it was shown that commercially made fermented products such as pickled cucumbers and fermented cucumber juice, sauerkraut and sauerkraut juice, beetroot juice, white borscht and żurek contained negligible amounts of vitamin B12. Despite the common conviction that there is no vitamin B12 in plant products, it turned out to be present in some, namely in sea-buckthorn jam and in pickled parsley juice. These are idiosyncratic but natural products that may not be to every consumer's taste, although they are in fact an alternative to supplements and to fortified food. We believe that it is worth continuing to search for plant-based sources of vitamin B12 so that this vitamin may be available to everyone regardless of the diet.

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