Assessment of in vitro bioaccessibility of pyridoxal, pyridoxine and pyridoxamine forms of vitamin B6 in various vegetables

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Summary
Vitamin B6 is defined as the sum of pyridoxal, pyridoxine and pyridoxamine vitamers. The content of each form is quite different in various types of vegetables. This investigation aimed to determine the amount and bioaccessibility of pyridoxal, pyridoxine and pyridoxamine vitamers of vitamin B6 in various vegetables. In the study, it was determined that the total vitamin B6 content in various vegetables (carrots, broccoli, brussels sprouts, zucchini, potatoes, cucumbers, red pepperoni, tomatoes, chard, green peppers, parsley, spinach, onions, green beans, leek and curly lettuce) ranged from 0.25 mg·kg^{-1} to 5.93 mg·kg^{-1}. The average proportion of pyridoxal, pyridoxine and pyridoxamine forms in vegetables was 25.9 %, 50.3 %, and 23.8 %, respectively. However, these proportions were 34.7 %, 34.7 % and 14.8 % in green leafy vegetables. The bioaccessibility of total vitamin B6 in samples ranged from 17 % to 69 %, and the average bioaccessibility of pyridoxal, pyridoxine and pyridoxamine vitamers was 43 %, 34 % and 51 %, respectively. The lowest and highest average bioaccessibility was determined for pyridoxine and pyridoxamine vitamers, respectively.

Keywords
bioaccessibility; pyridoxal; pyridoxine; pyridoxamine; vegetables; vitamin B6

Vegetables are an essential part of a healthy diet plan. Scientific evidence indicates a strong relationship between high vegetable consumption and the prevention of chronic diseases [1, 2]. Global health authorities recommend consuming three servings of vegetables per day for adults [3, 4]. However, it is reported that approximately 90 % of the USA population does not consume enough vegetables [3]. Vegetables are plant-based foods that vary widely in energy, nutrients and bioactive components. They contribute to diet quality because they have a low energy density and contain dietary fibre, carotenoids, vitamin C, potassium, calcium and folate. Vegetables, which essentially contribute to the quality and diversity of the diet, are also a good source of vitamin B6.

It is known that vitamin B6 is involved in essential biochemical reactions in human body, such as heme synthesis, neurotransmitters synthesis, steroid hormones activity, amino acid metabolism or glycogen breakdown. The active form, pyridoxal 5-phosphate (PLP), acts in more than 150 reactions in cellular biochemistry and metabolism. However, its central function is related to amino acid metabolism [5–7]. Therefore, its daily requirement is determined by dietary protein intake. Both animal- and plant-based foods, such as liver, meat, whole grain products, vegetables, potatoes or nuts, contain different amounts and forms of vitamin B6. Vitamin B6 can be found in foodstuffs in the form of pyridoxine (PN), pyridoxal (PL), pyridoxamine (PM), pyridoxine phosphate (PNP), pyridoxal phosphate (PLP), pyridoxamine phosphate (PMP) and pyridoxine glucoside

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(PNG). Common forms in animal-based foods are PL and PLP, while PN and PNG are common forms in plant-based foods [5, 6, 8].

The recommended daily intake is stated as 0.016 mg of vitamin B6 for 1 g of protein. A well-balanced diet plan provides the recommended daily intake level of vitamin B6 and, therefore, primary deficiency is rare [5, 6]. One of the best indicators of adequate dietary intake is serum PLP level [7]. Despite its widespread use, serum PLP level is an unreliable parameter as it is affected by inflammation, smoking, alcohol consumption and oral contraceptive use. Therefore, it is difficult to interpret the daily adequate dietary intake level for vitamin B6 [9, 10]. Due to physiological variability, the daily requirement of vitamin B6 may differ among individuals. It was also reported that the daily dietary intake level of vitamin B6 decreased in chronic alcoholics and the elderly [11, 12]. Although primary deficiency is reported to be rare, low dietary intake levels are associated with an increased risk of depression, metabolic syndrome and cardiovascular diseases [13, 14].

Dietary intake of vitamin B6 is a good predictor of PLP levels in the body. However, the dietary pattern also has the potential to affect the PLP status. Previous studies reported that bioavailability of the PLP vitamer in animal-based foodstuffs is higher than of the forms contained in plant-based foods [15, 16]. Therefore, poor protein digestibility and difficulties in deglycosylation of the intact PNG form may reduce the bioavailability of vitamin B6 in plant-based foodstuffs [6]. Bioavailability refers to the amount of nutrients that are absorbed in the digestive tract and reaches target tissues to perform their physiologic role [17]. While bioavailability is ideally studied in human volunteers, bioaccessibility of nutrients can be determined in vitro by simulating the human physiological state. Yaman [18] reported that bioaccessibility of vitamin B6 for PL, PN and PM vitamers in various bread types ranged from 56 % to 67 %. Bioaccessibility, an essential predictor of bioavailability, is stated as the amount of nutrients liberated from the food matrix in the gastrointestinal tract and ready for intestinal intake. Due to ethical restrictions, the number of studies measuring the digestibility of macronutrients and micronutrients in the gastrointestinal tract is insufficient. Hence, in vitro gastrointestinal investigations are preferred because they are cost-effective, short-term and do not present any ethical issues [19, 20].

Yaman and Mızrak [21] studied the bioaccessibility of three vitamers of vitamin B6 in cereal-based baby foodstuffs. However, the content and bioaccessibility of vitamin B6 in vegetables, an essential component of plant-based foods, has not been investigated yet. Our study aimed to determine the content and bioaccessibility of PL, PN and PM vitamers of vitamin B6 in various vegetables using a simulated gastrointestinal model.

MATERIALS AND METHODS

Chemicals

α-Amylase (1.5 $U \cdot mg^{-1}$, from *Aspergillus oryzae*, powder, EC 3.2.1.1), β-glucosidase (10–30 $U \cdot mg^{-1}$ solid, from almonds, lyophilized powder, EC 3.2.1.21), taka-diastase (100 $U \cdot mg^{-1}$, from *Aspergillus oryzae*, powder, EC 3.2.1.1), standards (pyridoxal·HCl, pyridoxine·HCl, pyridoxamine·2 HCl), acid phosphatase (0.5–3.0 $U \cdot mg^{-1}$, from potato, lyophilized powder, EC 3.1.3.2), NaCl, $CaCl_2 \cdot 2H_2O$, urea, uric acid, meta-phosphoric acid, KCl, mucin, lipase (100–500 $U \cdot mg^{-1}$ protein, from porcine pancreas, Type II, EC 3.1.1.3), bovine serum albumin, pepsin (≥ 250 $U \cdot mg^{-1}$ solid, from porcine gastric mucosa lyophilized powder, EC 3.4.23.1), bile salts mixture, pancreatin (from porcine pancreas), sodium acetate, $NaHCO_3$, acetonitrile, 1-octane sulfonic acid sodium salt, KH_2PO_4, HCl, trichloroacetic acid and ortho-phosphoric acid were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). All chemicals were of analytical grade.

Samples

In this investigation, 16 vegetables were studied. Samples of carrots, broccoli, brussels sprouts, zucchini, potatoes, cucumbers, red pepperoni, tomatoes, chard, green peppers, parsley, spinach, onions, green beans, leek and curly lettuce were purchased from local marketplaces in Turkey. Among the vegetables, broccoli, brussels sprouts, zucchini, potatoes, chard, spinach, green beans and leek were boiled in water before analysis. The boiling of vegetables was done at 100–102 °C (water temperature) for 15 min in a stainless steel pan and they were subsequently drained [22, 23].

Standards adjustment

Standard stock solutions were adjusted in 0.1 $mol \cdot l^{-1}$ HCl. Three levels of working standards were adjusted from the stock solution.

In vitro study

In vitro human gastrointestinal digestion was performed as described previously [21]. Composition of the salivary solution, gastric juice, duode-

Tab. 1. Composition of the saliva solution, gastric juice, duodenal juice and bile juice.

Solution	Volume	Chemical components		Enzymes		pH
Saliva	1000 ml	NaCl Urea Uric acid	3.4 ml (175.3 g·l^{-1}) 16 ml (25 g·l^{-1}) 30 mg	α-Amylase Mucin	580 mg 50 mg	7.0 ± 0.2
Gastric juice	1000 ml	HCl $CaCl_2 \cdot 2H_2O$ Bovine serum albumin	33 ml (37 g·l^{-1}) 36 ml (22.2 g·l^{-1}) 2 g	Pepsin Mucin	5 g 6 g	1.5 ± 0.1
Duodenal juice	1000 ml	KCl $CaCl_2 \cdot 2H_2O$ Bovine serum albumin	13 ml (89.6 g·l^{-1}) 18 ml (22.2 g·l^{-1}) 2 g	Pancreatin Lipase	18 g 3 g	7.5 ± 0.2
Bile juice	1000 ml	$NaHCO_3$ $CaCl_2 \cdot 2H_2O$ Bovine serum albumin Bile	136 ml (84.7 g·l^{-1}) 20 ml (22.2 g·l^{-1}) 3.6 g 60 g			7.0 ± 0.2

nal juice and bile juice employed in the in vitro gastrointestinal system are given in Tab. 1. These solutions and juices were adjusted by employing enzymes, organic and inorganic compounds. First, by using 1 000 ml of purified water as the solvent, organic and inorganic components were provided for each digestion mode. The enzymes and ingredients were then mixed based on the composition of every digestion mode. Finally, the pH value of individual media was adjusted to approximately pH 7, pH 1.5, pH 7.5, and pH 7 for the salivary solution, gastric, duodenal and bile juices, respectively.

The digestive modelling consisted of 3 phases. The initial step involved combining 5 g of the test sample and 5 ml of salivary solution, which was then incubated in a shaking water bath at 37 °C for 5 min. The next step involved the addition of gastric juice to the test sample and incubation in a shaking water bath at 37 °C for 2 h. The final step involved the addition of 10 ml of duodenal juice and 5 ml of bile juice to the sample from the gastric step, adjusting pH and incubation in a shaking water bath at 37 °C for 2 h.

The final amount of the test sample obtained from the digestion stage was added purified water to 50 ml. The pH value of the mixture was adjusted to pH 4.5 using trichloroacetic acid and then centrifuged at 8 000 ×*g* for 10 min. Next, 10 ml of the sediment was mixed with 10 ml of meta-phosphoric acid solution (30 g·l^{-1}). The solution was then filtered utilizing a cellulose acetate filter (pore size 0.45 μm).

After in vitro digestion, the enzymatic reactions were stopped by the addition of 1 mol·l^{-1} HCl and then content of vitamin B6 analysis was determined. Bioaccessibility was calculated by dividing the concentration of vitamin B6 in the digesta by the total vitamin B6 content in the original non-digested sample. The result was expressed as a percentage.

Vitamin B6 extraction and sample preparation

Vitamin B6 extraction was carried out as described previously [21]. First, the vegetables were homogenized and 5 g of the sample was put inside a 500 ml Erlenmeyer flask, 60 ml of 0.1 mol·l^{-1} HCl was added and the mixture was autoclaved for 30 min at 121 °C. The samples were cooled down to laboratory temperature (25 °C) and pH was adjusted to 4.5 by 2.5 mmol·l^{-1} sodium acetate. An enzymatic procedure was then conducted to liberate the phosphorylated vitamers of vitamin B6 (PLP, PMP and PNP). Following that, 100 mg taka-diastase, 10 mg acid phosphatase and 10 mg β-glucosidase were added to the test samples and they were incubated in a shaking water bath at 37 °C for 18 h. Next, the samples were cooled down to laboratory temperature (25 °C), their volume was adjusted to 100 ml with 0.1 mol·l^{-1} HCl, filtered through a cellulose acetate membrane filter (pore size 0.45 μm) and analysed by high-performance liquid chromatography (HPLC).

High-performance liquid chromatography

Vitamin B6 vitamers were determined by HPLC as described by Yaman and Mızrak [21] with some modifications. Nexera-i LC-2040C 3D pump and RF-20A fluorescence detector were employed (Shimadzu, Kyoto, Japan). The mobile phase was prepared by dissolving 11 g of KH_2PO_4 and 0.5 g of 1-octane sulfonic acid in 950 ml of deionized water. At that time, 50 ml acetonitrile was added and pH was adjusted to pH 2.4 using ortho-phosphoric acid. Excitation and emission

wavelengths were fixed at 290 nm and 395 nm, respectively. Zorbax Eclipse X08-C18 column (150 mm × 4.6 mm, 5 μm particle size; Agilent Technologies, Santa Clara, California, USA) was used. The flow rate was 0.8 ml·min^{-1}. and the column oven temperature was set at 25 °C.

Statistical analysis

Each analysis was done in triplicate and the mean value was calculated. Obtained data were subjected to a one-way analysis of variance to assess the PL, PM and PN contents of vegetable samples. Significance of the differences inside groups was evaluated by ANOVA and Tukey's test at $p < 0.05$.

RESULTS AND DISCUSSION

Vitamin B6 vitamers contents were determined for a range of vegetables prior to and after in vitro digestion. An HPLC chromatogram of PL, PN and PM forms of vitamin B6 in brussels sprout is shown in Fig. 1. In this method, each vitamer of vitamin B6 is determined separately and the total level of vitamin B6 is calculated. Statistically significant differences between samples prior to and after digestion were determined comparing the mean results of the contents of PL, PN, PM, and total vitamin B6 in all vegetables (Tab. 2). These results are consistent with levels of total vitamin B6 for the same vegetables ranging from 0.34 mg·kg^{-1} to 3.00 mg·kg^{-1} in the US Food Composition Database [24]. Vegetables, which are essential representatives of the diversity and quality of the diet, are a good source of vitamin B6. In an average Polish diet, potatoes and other vegetables represent 27.8 % of all dietary sources of vitamin B6 [25]. In Korean, Dutch and Spanish diets, these rates were reported as 28.7 %, 24.0 %, and 16.3 %, respectively [26–28]. These studies revealed the importance of adequate consumption of various vegetables in supplying the need for vitamin B6.

When foods of plant and animal origin are compared, the dominant chemical form of vitamin B6 tends to differ. It was reported that animal-based foods contain mainly PL and PM vitamers, while plant-based foodstuffs contain mainly PN vitamer [6, 15, 29]. In the literature, vitamin B6 is generally stated as the sum of the PL, PN and PM forms present in foods. However, identifying each form of vitamin B6 provides more specific and reliable data to clarify food composition. In our study, the average rate of PL, PN and PM vitamers was 25.9 %, 50.3 %, and 23.8 %, respectively. Our findings show that PN is the dominant form in vegetables, which is consistent with the literature data [6, 15, 29]. It was observed that the PN form and the PL form are dominant in green leafy vegetables. Also, the distribution of PL and PN forms in green leafy vegetables was found to be significantly different from that of other vegetables. While the average rate of PL and PN form was 34.7 % in green leafy vegetables, it was 13.7 % and 52.7 % in other vegetables, respectively. However, our results showed that PM was the dominant form in all peppers and the PM proportion in red and green peppers was 64.9 % and 70.1 %, respectively. Although it was stated that the dominant vitamer of vitamin B6 in vegetables is PN, we observed that the dominant form might differ in some vegetables.

Since approximately 4% of cellular enzymes are PLP-dependent, it has been established that vitamin B6 is an essential compound in human metabolism. Some studies reported that the PM vitamer of vitamin B6 inhibits protein glycation. ABDULLAH et al. [30] showed that the PM vitamer of vitamin B6 reduced the level of intracellular reactive oxygen species (ROS) induced by glycation of human serum albumin protein. Therefore, PM was described as a promising agent against progressive tissue damage in most chronic diseases [31, 32]. In a diabetic rat model, PM treatment was reported to suppress the production of advanced glycation end products (AGE) and to delay the development of diabetic nephropathy [33]. In phase 2 clinical trial, PM identified as an AGE-inhibitor was reported to have a protective function against diabetic nephropathy [34]. Therefore,

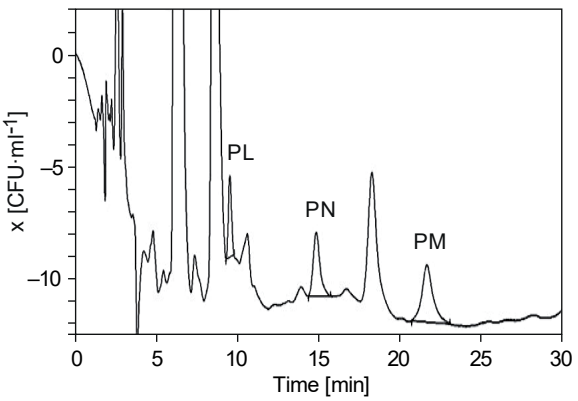

Fig. 1. HPLC chromatogram showing separation of pyridoxal, pyridoxine and pyridoxamine in brussels sprouts.

PL – pyridoxal, PN – pyridoxine, PM – pyridoxamine.

Tab. 2. Contents of vitamin B6 vitamers and total vitamin B6 in various types of vegetables.

Sample	Pyridoxal		Pyridoxine	
	Prior to digestion [mg·kg^{-1}]	After digestion [mg·kg^{-1}]	Prior to digestion [mg·kg^{-1}]	After digestion [mg·kg^{-1}]
Carrots	0.13 ± 0.01^{a}	0.05 ± 0.00^{b}	0.93 ± 0.04^{a}	0.34 ± 0.02^{b}
Broccoli*	0.27 ± 0.01^{a}	0.05 ± 0.00^{b}	0.14 ± 0.01^{a}	0.09 ± 0.01^{b}
Brussels sprouts*	0.61 ± 0.03^{a}	0.32 ± 0.01^{b}	0.77 ± 0.03^{a}	0.26 ± 0.01^{b}
Zucchini*	0.22 ± 0.01^{a}	0.13 ± 0.01^{b}	0.49 ± 0.02^{a}	0.16 ± 0.01^{b}
Potatoes*	0.13 ± 0.01^{a}	0.09 ± 0.00^{b}	0.81 ± 0.04^{a}	0.30 ± 0.01^{b}
Cucumbers	0.15 ± 0.01^{a}	0.03 ± 0.00^{b}	0.30 ± 0.01^{a}	0.04 ± 0.00^{b}
Red pepperoni	0.29 ± 0.01^{a}	0.10 ± 0.01^{b}	1.20 ± 0.05^{a}	0.55 ± 0.03^{b}
Tomatoes	0.29 ± 0.01^{a}	0.10 ± 0.01^{b}	0.69 ± 0.03^{a}	0.48 ± 0.02^{b}
Chard*	0.49 ± 0.02^{a}	0.32 ± 0.01^{b}	0.42 ± 0.02^{a}	0.10 ± 0.00^{b}
Green peppers	0.38 ± 0.02^{a}	0.28 ± 0.01^{b}	1.39 ± 0.06^{a}	0.62 ± 0.03^{b}
Parsley	0.71 ± 0.03^{a}	0.28 ± 0.01^{b}	1.91 ± 0.09^{a}	0.57 ± 0.03^{b}
Spinach*	1.06 ± 0.05^{a}	0.28 ± 0.01^{b}	0.80 ± 0.04^{a}	0.11 ± 0.01^{b}
Onions	0.18 ± 0.01^{a}	0.03 ± 0.00^{b}	0.57 ± 0.03^{a}	0.27 ± 0.01^{b}
Green beans*	0.03 ± 0.00^{a}	0.02 ± 0.00^{b}	1.18 ± 0.05^{a}	0.15 ± 0.01^{b}
Leek*	0.45 ± 0.02^{a}	0.36 ± 0.02^{b}	0.42 ± 0.02^{a}	0.14 ± 0.01^{b}
Curly lettuce	0.11 ± 0.00^{a}	0.04 ± 0.00^{b}	0.10 ± 0.00^{a}	0.03 ± 0.00^{b}

Sample	Pyridoxamine		Vitamin B6	
	Prior to digestion [mg·kg^{-1}]	After digestion [mg·kg^{-1}]	Prior to digestion [mg·kg^{-1}]	After digestion [mg·kg^{-1}]
Carrots	0.04 ± 0.00^{a}	0.02 ± 0.00^{b}	1.10 ± 0.05^{a}	0.41 ± 0.02^{b}
Broccoli*	0.20 ± 0.01^{a}	0.11 ± 0.01^{b}	0.61 ± 0.03^{a}	0.25 ± 0.02^{b}
Brussels sprouts*	0.77 ± 0.03^{a}	0.57 ± 0.03^{b}	2.15 ± 0.09^{a}	1.15 ± 0.05^{b}
Zucchini*	0.07 ± 0.00^{a}	0.04 ± 0.00^{b}	0.78 ± 0.03^{a}	0.33 ± 0.02^{b}
Potatoes*	0.31 ± 0.01^{a}	0.16 ± 0.01^{b}	1.25 ± 0.06^{a}	0.55 ± 0.02^{b}
Cucumbers	0.06 ± 0.00^{a}	0.02 ± 0.00^{b}	0.51 ± 0.02^{a}	0.09 ± 0.00^{b}
Red pepperoni	2.76 ± 0.12^{a}	2.30 ± 0.10^{b}	4.25 ± 0.18^{a}	2.95 ± 0.14^{b}
Tomatoes	0.48 ± 0.02^{a}	0.33 ± 0.02^{b}	1.46 ± 0.06^{a}	0.91 ± 0.05^{b}
Chard*	0.27 ± 0.01^{a}	0.11 ± 0.01^{b}	1.18 ± 0.05^{a}	0.53 ± 0.02^{b}
Green peppers	4.16 ± 0.19^{a}	3.16 ± 0.14^{b}	5.93 ± 0.27^{a}	4.06 ± 0.18^{b}
Parsley	0.43 ± 0.02^{a}	0.13 ± 0.01^{b}	3.05 ± 0.14^{a}	0.98 ± 0.05^{b}
Spinach*	0.18 ± 0.01^{a}	0.06 ± 0.00^{b}	2.04 ± 0.10^{a}	0.45 ± 0.02^{b}
Onions	0.07 ± 0.00^{a}	0.05 ± 0.00^{b}	0.82 ± 0.04^{a}	0.35 ± 0.01^{b}
Green beans*	0.14 ± 0.01^{a}	0.06 ± 0.00^{b}	1.35 ± 0.06^{a}	0.23 ± 0.01^{b}
Leek*	0.15 ± 0.01^{a}	0.07 ± 0.00^{b}	1.02 ± 0.05^{a}	0.57 ± 0.03^{b}
Curly lettuce	0.04 ± 0.00^{a}	0.02 ± 0.00^{b}	0.25 ± 0.00^{a}	0.09 ± 0.00^{b}

Values are mean ± standard deviation (n = 3). Different superscript letters in superscript in the same row indicate statistically significant difference between the value prior to digestion and after digestion for each form ($p < 0.05$)
* – vegetables boiled for 15 min.

dietary intake of vegetables with a high content and bioaccessibility of PM may be effective for preventing and managing AGE-related chronic diseases.

Foods mostly contain the protein-bound or glycoside form of vitamin B6 [6]. The complex form of vitamin B6 is converted to the free form by alkaline phosphatase and PNG hydrolase enzymes before intestinal absorption [6, 35]. In in vitro digestion models, phosphate and glycoside bonds are cleaved by enzymes and free forms of vitamin B6 (PL, PN and PM) are individually quantified. Subsequently, bioaccessibility values are calculated for each form [36].

In vitro digestion models are widely used to predict the bioaccessibility of nutrients due to their low cost, short duration and absence of ethical issues [19, 20]. However, no studies are available on the bioavailability of vitamin B6 from vegetables. Our results showed that in vitro bioaccessibility of vitamin B6 ranged from 17 % to 69 % (Tab. 3). YAMAN [18] reported that the bioaccessibility of vitamin B6 from grain products ranged between 56 % and 67 %. Consistent with the literature data, our study showed that plant-based foods have lower bioaccessibility of vitamin B6 [21, 37].

Most water-soluble vitamins are bound to polysaccharides or polypeptides and are released depending on gastric acidity. AKÇA et al. [38] reported that the bioaccessibility of vitamins B1, B2, and B3 at a gastric pH level of pH 4 was lower than at gastric pH level of pH 1.5 in cereal-based baby foods. The same was observed for the bioaccessibility of PL, PN and PM vitamers of vitamin B6 [21]. However, there is an inverse relationship between dietary intake of PNG and the bioavailability of vitamin B6 [39]. It was stated that the bioavailability of vitamin B6 in animal-based foodstuffs can reach up to 100 % [15]. GREGORY et al. [16] stated that the bioavailability of PNG, which is mainly found in vegetables, was 58 ± 13 %. In the present study, the average bioaccessibility of total vitamin B6 and PN vitamer was 41 ± 16 % and 34 ± 16 %, respectively. Previous studies attributed the low bioavailability of vitamin B6 in plant-based foods to the high fibre content of foods [21, 37]. It was also reported that the bioavailability of the free PN form may decrease with the presence of PNG in the diet [6]. Despite the potential for low bioavailability, there is insufficient evidence to suggest that vegans who consume an adequate and varied diet are at risk of vitamin B6 deficiency [40]. Primary deficiency

Tab. 3. Bioaccessibility of vitamin B6 vitamers and total vitamin B6 in various vegetables.

Sample name	Pyridoxal [%]	Pyridoxine [%]	Pyridoxamine [%]	Vitamin B6 [%]
Carrots	39 ± 2 ^{Fb}	37 ± 2 ^{Cb}	50 ± 2 ^{CDEa}	37 ± 3 ^{EFG}
Broccoli*	19 ± 1 ^{Hc}	65 ± 3 ^{A}	55 ± 3 Cb	41 ± 5 ^{DEF}
Brussels sprouts*	53 ± 2 ^{Eb}	34 ± 2 ^{CDc}	74 ± 3 ^{Ba}	53 ± 4 ^{C}
Zucchini*	59 ± 3 ^{DEa}	33 ± 1 ^{CDb}	57 ± 3 ^{Ca}	42 ± 9 ^{DE}
Potatoes*	69 ± 3 ^{BCa}	37 ± 2 ^{Cc}	52 ± 2 ^{CDb}	44 ± 3 ^{D}
Cucumbers	20 ± 1 ^{GHb}	13 ± 1 ^{Fc}	33 ± 2 ^{GHa}	18 ± 1 ^{H}
Red pepperoni	35 ± 2 ^{Fc}	46 ± 2 ^{Bb}	84 ± 4 ^{Aa}	69 ± $^{6 A}$
Tomatoes	35 ± 2 ^{Fb}	70 ± 3 ^{Aa}	69 ± 3 ^{Ba}	62 ± 6 ^{B}
Chard*	65 ± 3 ^{CDa}	24 ± 1 ^{Ec}	41 ± 2 ^{FGb}	45 ± 3 ^{D}
Green peppers	74 ± 3 ^{ABa}	45 ± 2 ^{Bb}	76 ± 3 ^{Ba}	68 ± 6 ^{AB}
Parsley	39 ± 2 ^{Fa}	30 ± 1 ^{Db}	30 ± 1 ^{Hb}	32 ± 3 ^{G}
Spinach*	26 ± 1 ^{Gb}	14 ± 1 ^{Fc}	33 ± 2 ^{GHa}	22 ± 2 ^{H}
Onions	17 ± 1 c	47 ± 2 ^{Bb}	72 ± 3 ^{Ba}	43 ± 3 ^{DE}
Green beans*	67 ± 3 ^{Ca}	13 ± 1 Fc	43 ± 2 ^{EFb}	17 ± 1 ^{H}
Leek*	80 ± 4 ^{Aa}	33 ± 2 ^{CDc}	47 ± 2 ^{DEFb}	56 ± 5 ^{C}
Curly lettuce	36 ± 2 ^{Fb}	30 ± 1 ^{Dc}	50 ± 2 ^{CDEa}	36 ± 0 ^{FG}

Values are mean ± standard deviation (n = 3). The uppercase letters in supercsript in the same column indicate statistically significant differences between samples, and lowercase letters in superscript in the same row indicate statistically significant differences between pyridoxal, pyridoxine and pyridoxamine forms of vitamin B6 of each sample ($p < 0.05$).
* – vegetables boiled for 15 min.

is rare, which is good because insufficient dietary intake would be associated with many health problems [13].

The bioavailability of vitamin B6 is affected by its stability, temperature, pH of the gastrointestinal tract and dietary fibre content of food [39]. EITTENMILLER et al. [41] revealed that the stability of the PN vitamer was higher than that of the PL and PM forms during heat treatment. In our study, 8 of 16 vegetables were boiled in water for 15 min to make them ready for consumption, whereas the bioaccessibility of our samples before boiling was not measured. Contrary to literature data, we observed that 7 out of 8 boiled vegetables had lower PN bioaccessibility than other forms. This may be because the intact PNG is not hydrolysed due to β-glucosidase denaturation [39]. However, it is inadequate to comment on this subject with limited data. Further investigations are needed on the bioaccessibility of vitamin B6 in boiled vegetables.

It is stated that all vitamers of vitamin B6 are stable in acidic solutions, but the stability of PN is higher than of other forms [41]. Similarly, YAMAN and MIZRAK [21] stated that PN bioaccessibility was higher than that of PL and PM at gastric pH 1.5 and pH 4 in cereal-based baby foods. The authors also suggested that the PN form may be more stable in alkaline environment and at high temperature. However, the PN vitamer had the lowest bioaccessibility (mean 34 ± 16 %), while the highest bioaccessibility was found in the PM vitamer (mean 51 ± 17 %) in our digestive model consisting of oral, gastric and intestinal phases. KUREK et al. [37] reported that adding dietary fibre to bread, due to the small fibre particle size, reduced the bioaccessibility of the PN form of vitamin B6. This suggests that the most stable vitamer of vitamin B6 in each food group may vary due to other bioactive compounds in its composition. We also observed that the bioaccessibility of PL, PN and PM vitamers ranged from 17 % to 80 %, from 13 % to 70 % and from 30 % to 84 %, respectively (Tab. 3). The wide range of values indicates that the bioaccessibility of the vitamers from each vegetable is highly variable.

CONCLUSIONS

This study provided data on the content and bioaccessibility of PL, PN and PM forms in carrots, broccoli, brussels sprouts, zucchini, potatoes, cucumbers, red pepperoni, tomatoes, chard, green peppers, parsley, spinach, onions, green beans, leek and curly lettuce. The content of vitamin B6 forms was quite different among various vegetables. Vegetables were found to contain mostly the PN form, although the dominant form of vitamin B6 in various vegetables may vary. Furthermore, our results showed that the bioaccessibility of vitamin B6 forms from various vegetable was quite different. The lowest and highest average bioaccessibility were determined for the PN and PM vitamers of vitamin B6, respectively.

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