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Research Article

Determination and evaluation in terms of healthy nutrition of the pyridoxal, pyridoxine and pyridoxamine forms of vitamin B_6 in animal-derived foods

Mustafa Yaman[1*]

[1]Department of Nutrition and Dietetics, Faculty of Health Sciences, İstanbul Sabahattin Zaim University, İstanbul, Turkey (ORCID: 0000-0001-9692-0204)

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Abstract

In many studies, vitamin B_6 is given as the sum of the pyridoxal (PL), pyridoxine (PN) and pyridoxamine (PM) forms. In a limited number of studies, PL, PN and PM forms of vitamin B_6 in animal origin foods were reported. Since the bioavailability of PL, PN and PM forms of vitamin B_6 are different; knowing the amounts of these forms in foods is important in terms of healthy nutrition. In this study, the PL, PN and PM forms in a total of 38 animal-based foods were determined by high performance liquid chromatography (HPLC). PL and PM were found predominantly in fish, meats and chicken samples. Within these, the highest amount of vitamin B_6 were found in golden grey mullet by 616.3 µg/100g, in veal fillet at 376.1 µg/100g and in chicken breast by 329.5 µg /100g, respectively. The PL form in total vitamin B_6 ranged in fish between 32.5 and 53.1%, in meats between 15.6 and 48.9%, and in chicken samples between 59.9 and 69.8%. It was also found that milk and milk products contain low amounts of vitamin B_6. Based on these results, all animal-based foods were found to be rich in terms of the PL and PM forms. The results of this study will play an important role in the creation of various diets for healthy nutrition.

Keywords: Vitamin B6 profile, animal-based foods, nutrition, HPLC

1. Introduction

Animal-based foods are rich in water soluble vitamins and important for healthy nutrition. Vitamin B_6 is one of the water-soluble vitamins and predominantly present in seven known forms in foods: pyridoxine (PN), pyridoxal (PL), pyridoxamine (PM), pyridoxine 5'-phosphate (PNP), pyridoxal 5'-phosphate (PLP), pyridoxamine 5'-phosphate (PMP), and PN-glycoside (PNG). After intestinal absorption, in the liver, all forms are converted into the PLP form by pyridoxine (pyridoxamine) 5-phosphate oxidase (Wozenski *et al*., 1980:Ball, 2004). The active form of vitamin B_6, PLP, plays an important role in amino acid metabolism and catalyzes deamination, transamination, decarboxylation, transsulfuration, and desulfurization reactions (Drewke and Leistner, 2001; Mittenhuber, 2001). Additionally, it plays an active role in the metabolism of neurotransmitters (dopamine, serotonin, glycine, glutamate, GABA) and the synthesis of nikotinamid adenin dinükleotid (NAD) from tryptophan (Ball, 2004).

The recommended daily intake of vitamin B_6 is between 1.5 and 1.7 mg for both males and females. In periods of pregnancy or lactation, however, this requirement is between 1.9 and 2 mg (Food and Nutrition Board, 1998). Vitamin B_6 is found in a wide variety of foods. Some of the best sources are meat, fish, wheat bran, grains, legumes and vegetables (USDA, 2018). Vitamin B_6 deficiency is associated with anemia, weakness, disorders of the digestive system, depression, confusion and visual disorders (Ball, 2004). Homocysteine is a sulfur-containing amino acid formed in the metabolism of methionine. PLP plays an important role in the conversion

* *Corresponding author* [1]Department of Nutrition and Dietetics, Faculty of Health Sciences, İstanbul Sabahattin Zaim University, İstanbul, Turkey, *ORCID: 0000-0001-9692-0204,* mustafayaman1977@gmail.com

of homocysteine into cysteine. Increased serum homocysteine levels in PLP deficiency can cause cardiovascular diseases (Miller *et al*., 1992). Recent studies have revealed that vitamin B_6 functions as a strong antioxidant in deactivating reactive oxygen species (ROS) created by oxidative stress in cells (Bilski *et al*., 2000). In epidemiological studies, it has been shown that PLP decreased the risk of colon and lung cancer (Mizushina *et al*., 1997; Slattery et al., 1997; Jansen *et al*., 1999).

Advanced glycation end products (AGEs), which are formed as a result of high levels of sugar in the blood, are thought to play an important role in the development of complications (nephropathy, neuropathy, retinopathy and atherosclerosis) related to diabetes (Sang and Young, 2018). Previous studies have reported that the PMP and PLP forms of vitamin B_6 inhibit AGEs formation (Nakamura and Niwa, 2005; Su-Yen and Mark, 2008). It has been stated that the daily vitamin B_6 requirement is 16 µg per gram of protein taken in the diet for both males and females. Accordingly, the vitamin B_6 requirement increases as the intake of protein increases (Miller *et al*., 1985; Hansen *et al*., 1996; Ball, 2004).

The PLP and PMP forms are predominantly found in animal-based foods while the PN-glucoside form is found in plant-based foods. The PN-glucoside form has a lower bioavailability than the PLP and PMP forms. The bioavailability of vitamin B_6 has been reported to be 58 ± 13% in the case of oral administration of the PN-glucoside form of vitamin B_6 to humans (Gregory *et al*., 1991). In a clinical study, when PLP and PN. hydrochloride (HCI) were administered orally to humans, serum PLP levels were reported to be approximately 50% higher in patients receiving PLP (Rossouw *et al*., 1978).

The high performance liquid chromatography (HPLC) method is preferred for the determination of vitamin B_6. Through this method, all forms of vitamin B_6 are determined (Mann *et al.*, 2001). Vitamin B_6 can be found in foods in free (PL, PN, PM), phosphate (PLP, PMP) or glycoside (PNG) forms. The bound forms are liberated by phosphatase and glycosidase enzymes into the free forms. In many studies, due to the matrix effects, the PL and PM forms are converted into the PN form by methods of derivation and the total vitamin B_6 result are given.

The PL, PN and PM forms of vitamin B_6 vary in animal-based foods. In many studies, in the literature or food composition databases, vitamin B_6 is given as the sum of the PL, PN and PM forms or in PN.HCI. In a limited number of studies, PL, PN and PM forms of vitamin B_6 in animal origin were reported. Therefore, knowing the rates of these forms is important for creating healthy diets for human nutrition,the aim of this study was to determine the PL, PN and PM forms of vitamin B_6 in fish, meat, chicken, egg, milk and milk products and evaluate these forms in terms of healthy human nutrition.

2. Materials and Methods

2.1. Mataerial

The vitamin standards (pyridoxal.HCI, pyridoxine.HCI, pyridoxamine.2HCI), acid phosphatase (EC 3.1.3.2) (potatoes, 0.5-3.0 U/mg), β-glucosidase(EC 3.2.1.21) (from almonds lyophilized powder, 10-30 units/mg solid), taka diastase (EC 3.2.1.1) (*Aspergillus Oryzae*, 100 U/mg), acetonitrile (ACN), potassium dihydrogen phosphate, and 1-octanesulfonic acid sodium salt were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A).

2.2. Sampling

All foods examined in this study were purchased from local markets. A total of 38 foods were examined from fish, meats (veal, beef, lamp, sheep), chicken, egg, milk and milk products.

2.3. Standard Preparation

A standard stock solution of each vitamin was prepared in a 0.1 N hydrochloric acid solution. The working standards on three levels were prepared from the stock solution.

2.4. Extraction of B_6 Vitamers in Animal-Based Foods

The extraction method described by Kall (2003) was used with some modifications. The samples were first homogenized, and a 5 g sample was put into a 250 ml Erlenmeyer flask. Next, 60 ml of the 0.1 N hydrochloric acid solution was added before the mixture was transferred to an autoclave where it was kept at 121°C for 30 minutes. The samples were cooled and then adjusted to pH 4.5 using a sodium acetate (2.5 mM) solution. 100 mg taka-diastase, 10 mg acid phosphatase, and 10 mg β-glucosidase enzymes were added. Then, it was incubated for 18 hours at 37°C in a shaking water bath. Afterwards, the samples were filtered with a 0.45 µm filter and transferred into the HPLC device. All analyses were performed in triplicate and the average value was used.

2.5. HPLC Determination of B6 Vitamers

The PL, PN and PM forms of vitamin B6 were determined by HPLC. The HPLC conditions described by Ceylan et al. (2018) were used with some modifications. The Shimadzu Nexera-i liquid chromatography system with a fluorescence detector (Shimadzu Corporation, Kyoto, Japan) was used in the study. The mobile phase was prepared by dissolving 11 g of KH2PO4 and 0.5 g of 1-octanesulfonic acid in 950 ml of deionized water. Then, 50 ml of ACN was added and the pH was adjusted to 2.4 with orthophosphoric acid. The fluorescence detector excitation and emission wavelengths were set at 290 and 395 nm, respectively. B6 vitamers were

separated with an Eclipse X08-C18, 5 µm, 4.6x150 mm column (Agilent, USA) with a flow rate of 0.8 mL/min. The column oven temperature was set to 25°C.

2.6. Quantification and Quality Control

In the study, certified reference material (Standard Reference Material 1849a: Infant Formula), was used to control the accuracy and the performance of the method. We also participated in a proficiency test for analysing breakfast cereal test material which was organized by FAPAS (Food Analysis Performance Assessment Scheme, UK, 2018).

3. Results and Discussion

The HPLC chromatogram of sheep shoulder is shown in Figure 1. As seen in the chromatogram, the PL, PN and PM forms of B_6 vitamers were well-separated in the sheep shoulder using the HPLC method. The retention times of PL, PN and PM forms were 7, 10.5 and 15 min, respectively. The stability of vitamin B_6, which is found naturally in foods, is lower than that of the synthetic form. Therefore, it is always recommended to use the quality control material for accuracy of the analysis. The amount of vitamin B_6 in the reference material was determined to be 13.21 mg/kg (assigned value 13.46 ± 0.93), and recovery was 98%. The FAPAS test result was found to be in the acceptable range ($-2 \leqslant$ Z score $\leqslant +2$).

The amounts of PL, PN and PM forms in vitamin B_6 and the total vitamin B_6 in fish are shown in Table 1. As seen in the table, the highest amount of vitamin B_6 was found in golden grey mullet by 616.3 µg/100g, and the lowest amount was found in anchovies by 122 µg/100g. In fish, the ratio of PL, PN and PM in vitamin B_6 was found to be between 32.5 and 53.1%, between 0 and 3.4 %, and between 46.9 and 67.5%, respectively. The average PL, PN and PM forms in fish were found at a ratio of 39.5%, 0.9% and 59.6%, respectively (Fig. 2a). The PL form was found at the highest ratio in red mullet, at 53.1%, while the lowest ratio was found in golden grey mullet and in European sea bass, at 32.5%. The PM form was found at the highest ratio in European sea bass and golden grey mullet, at 67.5%, while the lowest ratio was found in horse mackerel and red mullet, at about 47%. The PN form was found in a small amount in horse mackerel, turbot and European anchovy, at about %3. It is known that fish is a good source of protein, omega-3 fatty acids, vitamin D and water-soluble vitamins. As seen from our results, all fish are rich in both vitamin B_6 and in terms of the PL and PM forms, which are high bioavailable forms of vitamin B_6. Our total vitamin B_6 findings are consistent with the food composition databases (USDA, TURCOMP, DTU). However, in food composition databases and in the literature, the result of vitamin B_6 is given in total. In a limited number of studies, the vitamin B_6 profiles of fish are available. Ceylan *et al.* (2018) reported that gilthead sea bream contains 0.402 mg/100g of the total vitamin B_6 with high ratio of the PL form. In another study, the amount of vitamin B_6 was found in fresh salmon to be 0.509 mg/100g with high ratio of the PL form (Lebiedzińska *et al*., 2007).

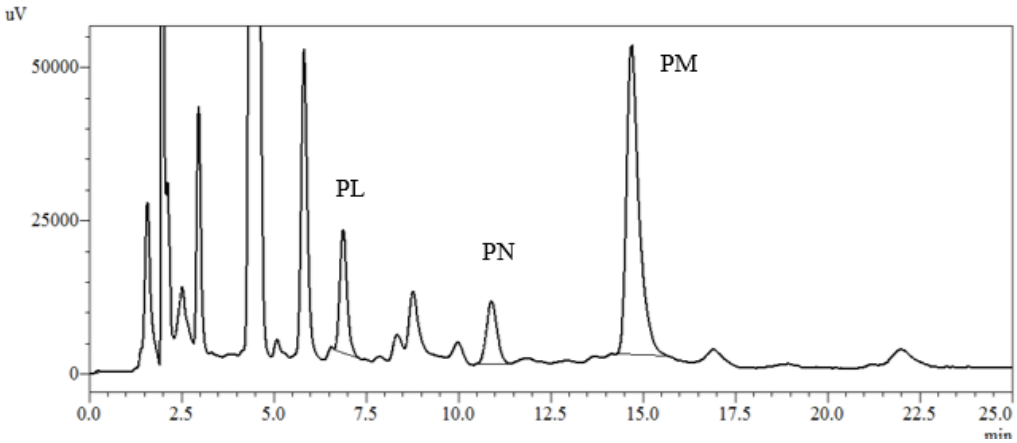

Figure 1. HPLC chromatogram of sheep shoulder

Table 1. Amounts of PL, PN and PM forms in vitamin B_6 and the total vitamin B_6 in fish.

Fish	PL µg/100g	PN µg/100g	PM µg/100g	Vitamin B_6, total µg/100g
Golden grey mullet	200.3±12.5	nd	416.0±26.3	616.3
Gilthead bream	140.4±8.7	nd	275.7±15.4	416.1
Bluefish	153.5±10.2	nd	197.5±10.0	351.1
European sea bass	108.4±6.5	nd	225.5±14.2	333.8
Horse mackerel	157.6±5.7	9.9±0.4	150.1±8.8	317.6
Red mullet	151.1±6.8	nd	133.3±10	284.4
European hake	90.3±6.0	nd	177.3±8.4	267.6
Rainbow trout	92.0±4.6	nd	154.3±12.2	246.2
Atlantic bonito	110.0±6.2	nd	130.5±7.5	240.5
Turbot	57.5±3.2	4.9±0.2	101.9±5	164.3
European anchovy	46.0±3.0	4.1±0.3	71.9±6.6	122.0

Average value was used (n=3), nd. not detected, pyridoxal (PL), pyridoxine (PN) and pyridoxamine (PM)

Table 2. Amounts of PL, PN and PM forms in vitamin B6 and the total vitamin B6 in veal, beef, sheep, lamb and chicken.

	PL µg/100g	PN µg/100g	PM µg/100g	Vitamin B6, total µg/100g
Veal				
Veal, fillet	183.9±9.4	24.7±2.1	167.5±12.7	376.1
Veal, leg	108.4±6.3	2.5±0.2	219.2±12.4	330.0
Veal, sirloin	46.8±2.3	24.7±1.5	248.5±14.8	320.0
Veal, rib	50.1±4.1	15.6±1.0	173.1±10.1	238.8
Veal, shoulder	46.0±2.8	11.5±0.6	180.1±9.5	237.6
Beef				
Beef, sirloin	56.6±3.5	28.0±1.3	252.0±15.5	336.6
Beef, shoulder	57.5±3.0	17.3±1.1	181.5±14.2	256.2
Beef, rib	77.2±4.4	28.8±1.4	141.7±7.5	247.7
Beef, fillet	76.4±4.2	10.7±1.0	145.9±4.9	232.9
Sheep				
Sheep, loin	37.8±2.0	7.4±0.5	107.5±6.6	152.7
Sheep, shoulder	51.7±3.2	24.7±0.8	111.7±7.3	188.1
Lamb				
Lamb, rib	67.3±4.4	23.9±1.2	125.6±10.1	216.8
Lamb, shoulder	35.3±2.3	41.2±1.6	106.8±6.0	183.2
Lamb, leg	67.3±4.1	0.8±0.1	85.2±2.2	153.3
Chicken				
Chicken, breast	229.9±13.4	8.2±0.3	91.4±4	329.5
Chicken, wing	183.1±11.1	29.6±2.0	74.0±4	286.7
Chicken, thigh	125.6±6.0	7.4±0.5	76.8±3.3	209.8

Average value was used (n=3), nd. not detected, pyridoxal (PL), pyridoxine (PN) and pyridoxamine (PM)

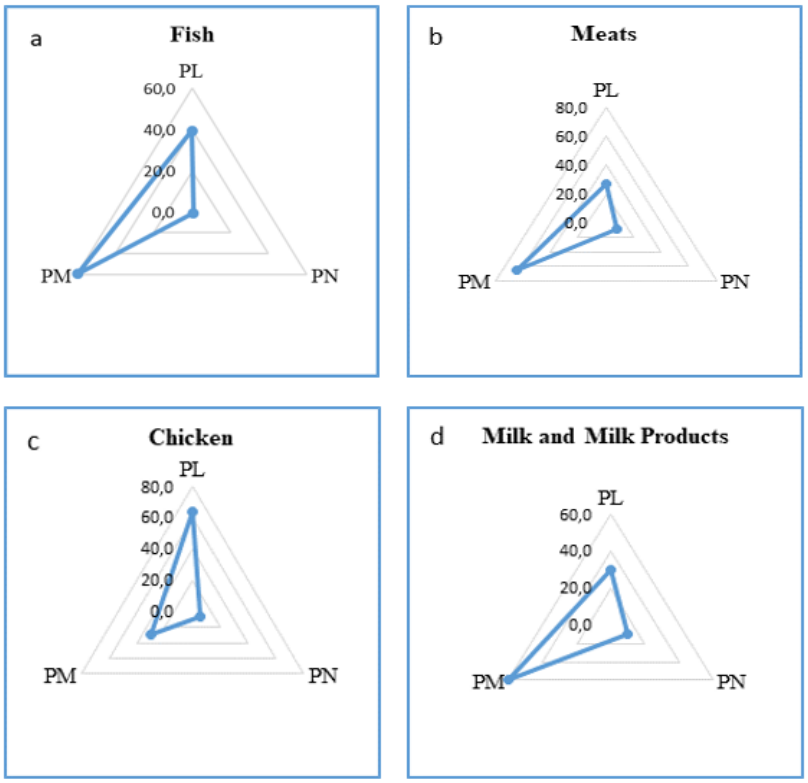

Figure 2. Rates of PL, PN and PM form in fish, meats, chicken and milk and milk products.

The amounts of PL, PN and PM forms in vitamin B_6 and the total vitamin B_6 in meats (veal, beef, sheep, lamb) and chicken samples are shown in Table 2. As seen in the table, in veal, beef, sheep and lamb, the total vitamin B_6 were found in the highest amount in veal fillet at 376.1 µg /100g, and the lowest amount was found in both leg of lamb and sheep loin at 153.3 µg /100g and 152.7 µg /100g, respectively. The PL form was found at the highest level in veal fillet, at 48.9%. The PN form was found in lamb shoulder at 22.5%, and the PM form was found in beef sirloin at 77.7%. In this meat group, the ratio of PL, PN and PM forms ranged between 14.6 and 48.9%, between 0.5 and 22.5%, and between 44.5 and 77.7%, respectively. The average PL, PN and PM forms were found at a ratio of 27.6%, 7.8% and 64.6%, respectively (Fig. 2b). As seen in the table, in veal and beef sirloin, the PL form was found at low rates (14.6-16.8%) while the PM form was found at high rates (74.9-77.7%).

In addition, when the ratio of PL form was evaluated in each meat, the highest difference was found in veal. The highest ratio of PL form was found in fillet, 48.9%, and the lowest ratio was found in sirloin, 14.6%. Kall (2003) reported that the total level of vitamin B_6 was found to be 350 µg /100g in beef (part of the animal unspecified) with a high ratio of the PL form (64%). In the same study, the PL form varied between 34 and 79% in pork meats. The total level of vitamin B_6 in cured hams was reported in the PN form (Gratacós-Cubarsí *et al.,* 2013). In vitamin B_6 analysis, the use of the phosphatase enzyme is required because the enzyme liberates the phosphatase bonds. Additionally, the enzymatic extraction time should be at least 18 hours to release the bonds in PLP, PNP and PMP. In some studies, either the enzyme or extraction time is not mentioned or not specified in the study (Esteve *et al*., 1998). Therefore, these conditions must be provided to obtain high quality results.

In the chicken samples, as seen in Table 2, the highest level of total vitamin B_6 was found in the chicken breast, 329.5 µg /100g, and the lowest level in the chicken thigh, 209.8 µg /100g. The ratio of PL, PN and PM forms in chicken samples were found to be between 59.9 and 69.8%, between 2.5 and 10.3%, and between 25.8 and 36.6%, respectively. The average PL, PN and PM forms in the chicken samples were found to be 64.5%, 5.5%, and 30.1%, respectively (Fig. 2c). Previous studies found that the sum of PM and PL in chicken was 80% of vitamin B_6 (Bowers and Craig, 1978) and the ratio of PM in raw chicken was 35% of all vitamin B_6 content (Olds *et al*., 1993). In our results, the average total sum of PL and PM in chicken was 94.5% of vitamin B_6 which is higher than that of other studies. As seen in our study, chicken contained a higher amount of the PL form than veal, beef, sheep and lamb. It was stated in other studies that the PLP form of vitamin B_6 was found mostly in meat, fish and poultry products; whereas the PN and PNP forms were found in (Ball, 2004; Ceylan *et al*., 2018). The PN and PNP forms of vitamin B_6 were predominant in plant-based foods (Gregor and Ink, 1987). Based on these results, it was found that the PL form was found at the highest level in the chicken samples, veal fillet, and leg of lamb compared to other meats, but the PN form was found at a lower level in all samples.

According to a study by Kall (2003), the PL and PM forms were mostly detected in meat and fish while the PN form was not found in these at all. Ceylan *et al*. (2018) reported that fish are rich in terms of both vitamin B_6 and its active form (PLP). In our results, the PM and PL forms were detected predominantly in fish, meat, and chicken while a very small amount of the PN form was found only in meats and fish.

As seen in Table 3, egg (whole) contain high amounts (240.8 µg/100g) of vitamin B_6, and the ratio of PN, PL and PM forms were found at the levels of 42.3%, 2.9% and 54.8%, respectively. Other milk and milk products contained low amounts of vitamin B_6. The highest amount was found in kashar cheese (55.2 µg/100g), and the lowest amount was found in UHT milk (10.7 µg/100g). Within the total vitamin B_6, the PL form was detected at the highest level in pasteurized cow's milk (61.5%) and not detected in UHT milk. The PN form was found at a low level in some milk and milk products. The PM form was detected at the highest level in sheep cheese (100%) and at the lowest level in pasteurized milk (26.2%). As seen, milk products contain high levels of the PM form (26.2-100%) of vitamin B_6 (Fig. 2d). We see that there is a loss of vitamin B_6 especially in cheeses as a result of production processes, and the PL form is completely lost in UHT milk and sheep cheeses. According to the study by Kall (2003), the amount of total vitamin B_6 was 50 µg/100g in skim milk and the ratio of PL and PM was 78% and 22%, respectively.

Table 3. Amounts of PL, PN and PM forms in vitamin B_6 and the total vitamin B_6 in egg, milk and milk products.

Sample name	PL µg/100g	PN µg/100g	PM µg/100g	Vitamin B_6, total µg/100g
Egg, chicken	101.8±5.5	7.0±0.5	132.0±9.5	240.8
Cheese, kashar ripened	18.9±1.2	nd	36.3±3.2	55.2
Cheese, kasar unripened	16.4±0.8	nd	24.4±1.8	40.9
Milk, sheep	10.7±0.6	10.7±0.7	17.5±1.1	38.8
Milk, cow	14.8±1.2	2.5±0.1	17.5±0.8	34.7
Cheese, sheep	nd	nd	32.8±3.3	32.8
Cheese (fat, 20 %)	11.5±0.7	5.8±0.3	14.7±1.2	31.9
Milk, pasteurised	8.2±0.4	1.6±0.1	3.5±2.0	13.3
Milk, UHT	nd	1.6±0.1	9.1±0.6	10.7

Average value was used (n=3), nd. not detected, pyridoxal (PL), pyridoxine (PN) and pyridoxamine (PM)

When we evaluated our results, fish, meat, chicken and milk generally contained high levels of the PL and PM forms and lower levels of the PN form. The bioavailability of the PL and PM forms of vitamin B_6 were higher than the PN form. In previous studies, it was reported that the bioavailability of vitamin B_6 of animal origin reached 100%, and fiber-containing foods reduced the bioavailability (Ball, 2004). It is known that vitamin B_6 has important roles in metabolism. The most important of these is that the PL and PM forms reduce the generation of AGEs (Su-Yen and Mark, 2008). In addition, in protein metabolism, the PLP form, in particular, of vitamin B_6 converts the homocysteine into the cysteine. It is recommended that 16 µg of vitamin B_6 per gram of protein should be consumed in the daily diet (Hansen *et al*., 1996; Ball, 2004). Although fish, egg, milk and other meats contain high amounts of protein (USDA, DTU), they also contain adequate amounts of vitamin B_6. Plant-based foods contain a high level of the PNG form of vitamin B_6. It was stated specifically that the bioavailability of foods containing the PNG form is very low. As is known, vitamin B_6 is essential for the metabolism of protein. As cereals and legumes contain high levels of protein; according to vitamin B_6 requirements, these foods should be consumed along with animal-based foods with high bioavailability. Additionally, processed milk products (cheeses, kashar cheese) contain high amounts of protein while they contain low amounts of vitamin B_6. Specifically, vitamin B_6 converts homocysteine into cysteine resulting from the methionine metabolism. Thus, serum homocysteine levels may increase vitamin B_6 deficiency. Therefore, other foods rich in vitamin B_6 may be consumed along with this group of foods.

4. Conclusion

In many studies in the literature and food composition databases, vitamin B_6 is given as the sum of the PL, PN and PM forms. This study is the most comprehensive profile-specification study of vitamin B_6 in animal-based foods so far. When we evaluated the results, the animal-based foods contained high amounts of vitamin B_6 as well as high levels of the PL form which is important in the metabolism of amino acids. Vitamin B_6 plays an important role in the metabolism of protein as well as in the reduction of the levels of serum AGEs and homocysteine. The occurrence rates of the PL, PN and PM forms of vitamin B_6 vary based on animal groups. This profile determination study will be an important source for various diets.

Acknowledgments

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