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*Review article*

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# SOURCES OF FOLATES IN HUMAN DIET

# ŹRÓDŁA FOLIANÓW W DIECIE CZŁOWIEKA

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**Streszczenie.** Najwyższą zawartością folianów charakteryzuje się żywność pochodzenia roślinnego. Niestety, związki te są podatne na utlenianie; nieunikniony jest również ich termiczny rozkład oraz wymywanie wodą. W świeżych produktach spożywczych poddanych obróbce termicznej pozostaje od około 40 (brokuły, soczewica) do ponad 90% (cebula, soja, jajo kurze) folianów. W poddanych obróbce termicznej mrożonkach zawartość folianów jest jeszcze mniejsza: straty podczas mrożenia wynoszą 10–20%, w trakcie magazynowania wzrastają dodatkowo o 16% do prawie 100%. Ponadto przyswajalność folianów ograniczają inhibitory koniugazy obecne w warzywach (kapusta, pomidory, fasola) i pomarańczach, a także polifenole (wino, piwo, herbata, owoce). W produktach pochodzenia zwierzęcego (z wyjątkiem wątroby) zawartość folianów jest średnio 2–2,5-krotnie mniejsza niż w warzywach. Jednak charakteryzują się one znacznie większą stabilnością ze względu na obecność lipofilnych antyoksydantów, a w przypadku produktów mleczarskich również białka wiążącego foliany (FBP). Wolniejsze tempo wchłaniania folianów związanych z białkiem oraz ochrona przed bakteriami jelitowymi zwiększa ich biodostępność. Absorpcja i metaboliczne wykorzystanie folianów jest większe w przypadku produktów zwierzęcych niż roślinnych.

**Key words:** bioavailability, folates, food products, stability.
**Słowa kluczowe:** biodostępność, foliany, produkty spożywcze, stabilność.

## INTRODUCTION

The aim of this study was to present the role folates play in human nutrition, taking into account their bioavailability and potential dietary supplements. While presenting food products which are a good source of folates, the impact of technological processes on changing their contents was taken into account. Folate deficiency is common in the diet of the Poles, which results from its low stability in long-term stored food. Folates occur in food in the form of 40 compounds, reduced derivatives of pteroylglutamic acid (PGA) – tetrahydrofolate (TH4) or dihydrofolate (DH2), in which glutamic acid residues are attached to pteroyl residue. Pteroylmonoglutamic ring (PteGlu), characteristic of folic acid is fully oxidized, and occurs naturally in trace amounts; a pteridine base, p-aminobenzoic acid and glutamic acid can be distinguished in its structure. Originally, folates were isolated from spinach leaves, therefore their name is derived from the word "folium", meaning a leaf (Cieślik and Kościej 2012).

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**Folates – importance for health**

Folates are involved in the synthesis of purine and pyrimidine bases, as well as nucleic acids. Therefore, they play an important role in the synthesis and methylation of DNA, determine its integrity and stability, thus preventing the development of many diseases, including cancer. Moreover they perform vital functions in the metabolism of amino acids, e.g. in the process of remethylating homocysteine to methionine, preventing hyperhomocysteinemia. The active form of folic acid is tetrahydrofolate, which is involved as a coenzyme in the transfer of mono-carbon residues (groups: methyl, methylene, formyl and formin) in the synthesis of purines, pyrimidines (thymine), certain amino acids (e.g. homocysteine), as well as in the conversion of histidine to glutamate. Alongside vitamin $B_{12}$ it participates in the formation and maturation of red blood cells. It is essential for the development of cells with intense division process, including fetal cells (Czeczot 2008). Deficiencies of folic acid are the cause of developmental disorders in the fetus (including neural tube defects); the DNA synthesis and cell division are delayed, which may lead to megaloblastic anaemia. Folic acid plays an important role in myelination of nerve fibers, preventing numerous neurological disorders (Kruman et al. 2002). Pteridine is involved in the synthesis of catecholamine neurotransmitters such as dopamine, adrenaline and serotonin, and in the form of BH4 (tetrahydrobiopterin) is a cofactor of phenylalanine, tyrosine and tryptophan monooxygenase (Czyżewska-Majchrzak and Paradowska 2010). By affecting the increase in the concentration of homocysteine in the blood, folate deficiency promotes the formation of blood clots and the development of atherosclerosis and its clinical implications such as hypertension, heart attack or stroke (Dierkes et al. 1998). Furthermore, folic acid deficiency promotes development of colorectal cancer and other tumours.

**Standards of folic acid intake**

The demand for folates depends on age, gender and physiological state. Standards for folic acid for adults and children over one year of age are expressed as EAR – Estimated Average Requirement for a group and RDA – the value sufficient to meet the demand for folic acid of approx. 97.5% of people in the group. However, standards for folic acid for infants are set at an adequate intake (AI) for all children under 1 year of age. Recommended daily intake of folate increases for pregnant women and nursing mothers and is 600 and 500 micrograms of dietary folate equivalents per person a day respectively (Table 1). The demand for folic acid is expressed in micrograms of dietary folate equivalents (DFE), which corresponds to 1 microgram of folates naturally found in foods, which in turn corresponds to 0.5 micrograms of folic acid from supplements taken on an empty stomach or 0.6 micrograms of folic acid consumed with food (Jarosz 2012). According to many authors the bioavailability of folate from food at the level 50% of bioavailability of synthetic folic acid is underestimated. During a 4-week study on the bioavailability of folates from food, it was found that it amounts to 78% (method using isotope) and 85% (based on the change in the concentration of folates in serum) in relation to folic acid (Winkels et al. 2007). Similarly, the bioavailability of folates in relation to folic acid evaluated on the basis of concentration of folates in serum amounts to 78%, in erythrocytes 98% and only 68% when evaluated on the basis of the concentration of homocysteine (Brouwer et al. 1999).

Table 1. Standards of folic acid intake
Tabela 1. Normy spożycia dla kwasu foliowego

| Population group<br>Grupa populacyjna<br>Age [Years]<br>Wiek [Lata] | µg dietary folate equivalents – person / day<br>µg równoważnika folianów – osoba / dzień | | |
|---|---|---|---|
| | EAR | RDA | AI |
| Babies<br>Niemowlęta | | | |
| 0–0.5 | | | 65 |
| 0.5–1 | | | 80 |
| Children<br>Dzieci | | | |
| 1–3 | 120 | 150 | |
| 4–6 | 160 | 200 | |
| 7–9 | 250 | 300 | |
| Boys and girls<br>Chłopcy i dziewczęta | | | |
| 10–12 | 250 | 300 | |
| 13–15 | 330 | 400 | |
| 16–18 | 330 | 400 | |
| Men and women<br>Mężczyźni i kobiety | | | |
| >19 | 320 | 400 | |
| Pregnancy<br>Ciąża | 520 | 600 | |
| Lactation<br>Laktacja | 450 | 500 | |

Source: – Źródło: Bułhak-Jachmyczyk 2008.

Due to widespread fortification of food with synthetic folic acid and popularity of dietary supplementation, especially among women of childbearing age, new maximum tolerable intake has been set (UL – upper level), which for adults is 1 mg/day (Berry et al. 2010). UL refers to the intake of folic acid only from supplements and fortified food. Exceeding this dose can cause progression or occurrence of neuropathy in patients with deficiency of vitamin $B_{12}$. It may also mask cobalamin deficiency and anaemia (Sweeney et al. 2007). In people with known tumour lesions folate deficiency can slow or inhibit the process of carcinogenesis. However, excess of folates – due to their participation in the synthesis of DNA – can lead to accelerated pace of cancer cells replication, it can also cause intensified inflammation and consequently, development and destabilization of atherosclerotic plaque. Several studies have shown that high doses of synthetic folic acid may increase the possibility of cancer (Charles et al. 2004, Kim 2004, Ebbing et al. 2009). Furthermore, unmetabolized folic acid can have immunosuppressive effect, reducing activity and cytotoxicity of NK cells (natural killers) (Sawaengsri et al. 2013). The form in which folates are delivered to the body has a major impact on the functioning of tissues and organs. Unfortunately, synthetic folic acid has higher affinity for membranes than folates naturally present in food. For this reason, transportation to cells and application in the body may be ineffective (Czyżewska-Majchrzak and Paradowska 2010).

**Folate intake in polish population**

Deficiency of folic acid is one of the most common hypovitaminosis in Poland. Even a well-balanced diet does not cover the demand for folic acid. As a result, as many as 90% of women and 78% of men do not meet daily requirements for folic acid (Waśkiewicz et al. 2010).

The study conducted by Stefańska et al. (2009) shows that depending on body weight, women covered only 55–63% of demand for folic acid. In contrast, the study of diets of women from the Małopolskie province shows that 78% of them do not cover the demand for this vitamin (Bieżanowska-Kopeć et al. 2007). Folic acid intake is also quite low among men – men from Warsaw meet only 48% of demand for folic acid (Dybkowska et al. 2007). Deficiency of folic acid was also noted in the diet the elderly (aged 60–96) – women covered only 60%, while men 72% of the demand (Stawarska et al. 2009). Long term deficiency of folates in the diet is one of the reasons for the increasing incidence of neurological and neurodegenerative disorders. For this very reason the supplementation is necessary. This applies especially to women planning to conceive and those already pregnant. The study conducted by Hamułka et al. (2003) shows that folic acid supplementation before pregnancy applied only to 24% of the women surveyed. More recent studies found that folic acid supplements were used by approximately 88% of pregnant women and 33% of women planning conception (Suliga 2011). In the study led by Cieślik et al. (2013) involving 268 women aged 20–45, only 58% used supplements, despite the fact that most of the participants were aware that folic acid is essential for proper development of the foetus. However, meeting the demand for folic acid is not always equal to its metabolic utilization. Metabolism of folic acid takes place with the participation of the reductase enzyme 5,10-methylene-TH4-folate (MTHFR) to 5-methyl-TH4-folate. Unfortunately, patients with MTHFR gene polymorphism have reduced activity of this enzyme. Reductase activity is essential in remethylation of homocysteine to methionine, the consequence of which is increased concentration of homocysteine (Barua et al. 2014). Research by Seremak-Mrozikiewicz et al. (2013) conducted with 1258 women and 68 men showed that the frequency of genotypes 677CT and 677TT in women is respectively 42.8% and 7.8%. Similar frequency of both genotypes was found in men, 41.2% and 7.3% respectively. In people with decreased MTHFR activity, getting folic acid, even at a recommended dose, may be ineffective in the prevention of defects in the central nervous system. In addition to folic acid, people with gene 677C> T MTHFR polymorphism should use – requiring no transformation – metabolically active form of folic acid – 5-methyl-TH4-folate. This compound (in the form of the calcium salt) is available as a dietary supplement – Metafolin® (*L-5-MTHF*-Ca). Both folic acid and metafolin are characterized by comparable physiological effects and bioavailability. Metafolin is also used instead of folic acid in food fortification (Pietrzik et al. 2010). A significant difficulty in estimating folate intake is their diverse bioavailability and low stability in food during storage, especially during the culinary processing. It is known that loss during storage and heat treatment can reach up to 90%. Moreover, commonly used food preservatives (benzoate, sulfites) inactivate all the B vitamins, including folates in the gastrointestinal tract. Prevention of neurological and neurodegenerative disorders seems impossible without supplementing diet with folic acid.

### Food sources of folate in human diet

Study on consumption of different products shows that the main source of folate in diet of the Poles are vegetable products (over 65%) – especially cereal (40%) and vegetables (25%). Animal products provide far less folate (Wartanowicz 1997). Leafy vegetables, legumes, nuts, whole grains and fruit are rich sources of folate. Even in Northern European countries, where consumption of plant foods is relatively low, it provides more than 50% of the daily intake of folate (Becker 2000). The animal food folate content is much lower because the animals do not produce p-aminobenzoic acid, which is necessary for the synthesis of the acid. The chicken liver as well as beef and veal liver are the exception, due to the fact that folates are accumulated primarily in the liver. The content of folates in the above products is as follows: 590, 330 and 240 μg · 100 $g^{-1}$. Egg yolk is also a good source of folate (152 μg · 100 $g^{-1}$) (Table 2). Small amount of folate is synthesized by yeast and micro flora colonizing the digestive tract in both humans and animals (Camilo et al. 1996). Among the fish, the best source of folic acid is salmon (26 μg · 100 $g^{-1}$). Folates in animal products (as opposed to plants) feature high stability. This is mainly due to the presence of lipophilic antioxidants (α-tocopherol, β-carotene, vitamin A and $D_3$, Coenzyme Q10), and in the case of dairy products also the presence of CLA and folate binding protein (FBP). Milk is not a good source of folates (5–12 μg · 100 $g^{-1}$), but they are characterized by high bioavailability thanks to fusion with whey proteins. Because of numerous strains of lactic acid bacteria's ability to synthesize folates, yogurt and buttermilk contain significantly more folates than milk. Hard cheeses are characterized by lower levels of folates (10–40 μg · 100 $g^{-1}$) than soft cheeses. The most folates (50–100 μg · 100 $g^{-1)}$ are found in soft moldy cheese due to lower whey syneresis (than in the case of hard cheese), as well as because of microbial synthesis of folates during ripening (Kowalska and Cichosz 2014). Folates in dairy products feature high stability, which results from the presence of hydrophilic antioxidants (vitamin C, glutathione, enzymes, group of sulphur amino acids – SH) as well as heat stable lipophilic antioxidants (CLA, α-tocopherol, β-carotene, vitamin A and $D_3$, Coenzyme $Q_{10,}$ phospholipids). Although lipophilic antioxidants are present in small amounts, regeneration of some at the expense of others is possible through synergistic interactions between them as well as between hydrophilic antioxidants. Thanks to this, antioxidants prevent the oxidation of folates during heat treatment of milk and storage of dairy products. Unfortunately, high heat treatment of milk (in the production of yogurt, for example) affects the denaturation of both folates and folate binding protein (FBP). Furthermore, ability to bind folate by FBP is reduced and so is their bioavailability (Wigertz et al. 1996). Nevertheless, yogurts, as well as other fermented milk drinks, contain more folates than milk, as a result of the synthesis of folic acid by various strains of lactic acid bacteria. The high bioavailability of folates from milk and dairy products, results from the fact that they appear mainly in the form of easily digestible monoglutamates (there is approx. 3 times more of them than polyglutamates). Present in milk and soya beans, monoglutamates do not require enzymatic transformations (deconjugation), unlike the 6- and 7-glutamic compounds present in the cabbage, for example (Ziemlański and Wartanowicz 2001). In addition, milk contains folate binding protein FBP facilitating transport of folates through cell membranes. Activity of FBP is increased by cholesterol and sphingolipids present in milk fat. Regular consumption of milk and dairy products – due to the presence of vitamin $B_{6,}$ $B_{12}$ and folic acid – lowers the concentration of homocysteine in blood serum (as has been demonstrated in numerous scientific studies and epidemiological studies) (Ganii and Kafai 2004, Lutsey et al. 2006).

Table 2. Folates content in animal and plant food
Tabela 2. Zawartość folianów w produktach pochodzenia zwierzęcego i roślinnego

| Products of animal origin<br>Produkty pochodzenia zwierzęcego | | Products od plant origin<br>Produkty pochodzenia roślinnego | |
|---|---|---|---|
| | (µg · 100 g$^{-1}$ or – lub 100 ml) | | |
| Chicken liver – Wątróbka z kurczaka | 590 | Spinach – Szpinak | 193 |
| Beef liver – Wątroba wołowa | 330 | Bean, dry seeds – Fasola biała, nasiona suche | 187 |
| Calf liver – Wątroba cielęca | 240 | Parsley Root – Pietruszka korzeń | 180 |
| Hen egg yolk – Żółtko jaja kurzego | 152 | Parsley leaves – Pietruszka liście | 170 |
| Pork liver – Wątroba wieprzowa | 110 | Brussels sporut – Brukselka | 130 |
| Hen egg – Jajo kurze całe | 65 | Kale – Jarmuż | 120 |
| Brie, camembert – Ser brie, camembert | 62 | Broccoli – Brokuły | 119 |
| Skim milk powder – Odtłuszczone mleko w proszku | 50 | Muesli nuts and raisins – Musli z rodzynkami i orzechami | 100 |
| Whole milk powder – Pełne mleko w proszku | 40 | Lettuce – Sałata | 75 |
| Rokpol – Ser Rokpol | 37 | Green bean – Fasola szparagowa | 70 |
| Pâté – Pasztet pieczony | 28 | Chive – Szczypiorek | 64 |
| Quark – Sery twarogowe | 27 | Avocado – Awokado | 62 |
| Turkey breast – Mięso z piersi indyka | 26 | Brown rice – Ryż brązowy | 59 |
| Salmon (fresh, smoked) – Łosoś (świeży, wędzony) | 26 | Cabbage – Kapusta biała | 57 |
| Hen egg white – Białko jaja kurzego | 20 | Leek – Por | 56 |
| Edam cheese – Ser edamski | 20 | Cauliflower – Kalafior | 55 |
| Cottage cheese – Serek ziarnisty | 15 | Wheat flour type 500 – Mąka pszenna typ 500 | 54 |
| Duck's carcass – Kaczka tuszka | 13 | Cucumber, red pepper – Ogórek zielony, papryka czerwona | 52 |
| Chicken breast – Mięso z piersi kurczaka | 12 | Whole grain rye bread – Chleb żytni pełnoziarnisty | 44.8 |
| Codfish – Dorsz świeży | 12 | Garham braed, tomato – Chleb graham, pomidor | 39 |
| Plain yoghurt 2% of fat – Jogurt naturalny 2% tł. | 10 | Beetroots – Buraki | 37 |
| Beef (tanderloin) – Wołowina (polędwica) | 10 | Buckwheat, carrot – Kasza gryczana, marchew | 32 |
| Herring – Śledź | 9 | White rice – Ryż biały | 29 |
| Sweetened condensed milk – Mleko zagęszczone słodzone | 9 | Wheat rolls – Bułki pszenne zwykłe | 28.4 |
| Chicken carcass – Kurczak tuszka | 8 | Oranges, red radish – Pomarańcze, rzodkiewka | 24 |
| Unsweetened condensed milk – Mleko zagęszczone niesłodzone | 8 | Bananas – Banany | 22 |
| Strawberry yoghurt 1.5% of fat – Jogurt truskawkowy 1,5% tł. | 6.9 | Strawberries, onion – Truskawki, cebula | 17 |
| Veal, goose – Cielęcina, gęś | 5 | Dried apricots – Morele suszone | 14 |
| Milk (0.5% of fat; 3.5% of fat) – Mleko spożywcze (0,5 tł.; 3,5% tł.) | 5 | Gooseberries, lemnos – Agrest, cytryny | 12 |
| Kefir – Kefir | 5 | Apples, blueberries – Jabłka, jagody | 6 |
| Cream 30% of fat – Śmietanka kremówka 30% tł. | 4 | Apricots, pineapple – Morele, ananas | 5 |
| Pork meat (loin and shoulder) – Mięso wieprzowe (schab i łopatka) | 3 | Tangerines, raspberries, plums – Mandarynki, maliny, śliwki | 3 |
| Butter – Masło | 3 | Strawberry jam – Dżem truskawkowy | 1.6 |
| Whey – Serwatka płynna | 1 | Rapeseed oil, sunflower oil – Olej rzepakowy, słonecznikowy | 0 |

Source: – Źródło: Dierkes et al. 1998, Kunachowicz et al. 2005, Owens et al. 2007, Chew et al. 2012.

**Food fortification with folic acid**

Fortified food is becoming increasingly important for meeting folic acid daily requirement. Despite the fact that in Poland fortifying food with folic acid is not mandatory, market of enriched products continues to expand. The maximum amount of folic acid that can be added to foods is defined in the Regulation of the Minister of Health dated 16 September 2010 on enriching substances added to food. The maximum permissible amount of folates per 100 g, 100 ml or per portion of the product must not exceed 100% of the recommended daily intake established for the purposes of food labelling, i.e. 200 µg. As part of the prevention of neural tube defects, almost 50 countries have established mandatory fortification of selected foods with folic acid (Sicińska et al. 2013). Since 1998 it has been mandatory in the United States to enrich white flour with folic acid in the amount of 140–150 mg $\cdot$ 100 $g^{-1}$ (Bailey 2010). The result is a 19% reduction in neural tube defects (Honein et al. 2001), as well as lower risk of hyperhomocysteinemia (Hertrampf et al. 2003, Kim 2004). Among the products fortified with folic acid available on the Warsaw market, there were mostly cereals (40%), juices, nectars and fruit drinks (27%), as well as sweets (12%) and instant drinks (9%) (Sicińska 2011). Unfortunately, the content of folic acid in fortified foods do not always follow the value declared by the manufacturer on the packaging. The folic acid content was in accordance with the manufacturer's declaration in just 1 out of the 6 juices purchased in Olsztyn's market. Because during three months of storage (20–22ºC and 5–7ºC) there was no significant reduction in folic acid content in juices, it must be stated that declarations from the manufacturers were untrue (Gujska et al. 2013).

**Stability of folates in food**

5-methyl-TH4-folate and 10-formyl-TH4-folate present in food can be converted in technological processes into inactive forms, i.e. into 5-methyl-5.6-TH4-folate and 10-formylofolate acid. Aa much as 50% of the total content of folates found in processed foods is a 5-methyl--5.6-TH4-folate (Czeczot 2008). Folates are extremely susceptible to oxidation, the consequence of which is their decomposition into inactive forms, principally p-aminobenzoyl-glutamate. The pace of reaction depends on the pH and buffering capacity of the environment, as well as the presence of antioxidants (Forssén et al. 2000, McKillop et al. 2002). Regardless of oxidation, during cooking food the folates are eluted with water, followed by thermal decomposition (Table 3). During cooking spinach and broccoli in water the loss of folates was 51% and 56% respectively, when compared to the initial content; however, after steaming such loss was not observed. Cooking whole potatoes for 60 minutes also resulted in no loss in folates (McKillop et al. 2002, Lešková et al. 2005). In Czech study different losses in 5-methyl-TH4-folate were noted depending on the type of vegetables. After 8 minutes of boiling spinach, Italian lettuce and carrots from 48% to 63% of folates were reduced and in Brussels sprouts, cauliflower and broccoli loss was lower and amounted to approximately 25% of the initial 5-methyl-TH4-folate. Differences in losses of folate may result from the properties of vegetables, such as weight to surface ratio and the presence of antioxidants in particular. In general, in leafy vegetables folate losses are greater than in non-leafy vegetables (Holasová et al. 2008).

Table 3. The influence of heat treatment on folates content in food
Tabela 3. Wpływ obróbki termicznej na zawartość folianów w żywności

| Product<br>Produkt | Folate content<br>Zawartość folianów<br>μg · 100 g⁻1 product<br>μg · 100 g⁻1 produktu | | Retention<br>Retencja<br>[%] |
|---|---|---|---|
| | fresh<br>surowy | after heat treatment<br>po obróbce | |
| Broccoli*<br>Brokuły* | 56 ±3 | 25 ±1 | 47 |
| Spinach*<br>Szpinak* | 251 ±2 | 152 ±2 | 64 |
| Onion*<br>Cebula* | 17 ±0 | 16 ±0 | 90 |
| Liver**<br>Wątroba** | 198 ±21 | 30 ±3 | 14 |
| Hen eggs***<br>Jaja kurze*** | 190 ±5 | 188 ±13 | 98 |
| Yolk***<br>Żółtko*** | 354 ±1 | 332 ±7 | 97 |
| Lentil****<br>Soczewica**** | 83 ±2 | 25 ±0 | 43 |
| Soybean****<br>Soja**** | 101 ±1 | 77 ±0 | 95 |
| Potatoes*****<br>Ziemniaki***** | 76 ±9 | 57 ±5 | 74 |

* boiled in a covered saucepan (100 ±2 g in 500 ml water, 10 min total cooking time) – gotowano w przykrytym garnku (100 ±2 g w 500 ml wody, 10 min całkowity czas gotowania);
** roasted in a sheet (100 ±2 g at 180ºC for 30 min) – pieczono (100 ±2 g w 180°C przez 30 min);
*** boiled in a covered saucepan (4 eggs / yolks in 250 ml water, 10 min total cooking time) – gotowano w rondlu z przykrywką (4 jaja / żółtka w 250 ml wody, 10 min całkowity czas gotowania);
**** soaked (100 ±2 g in 300 ml water at room temperature for 16 h) and boiled (100 ±2 g in 400 ml water, 25 min total cooking time) – moczono (100 ±2g / 300 ml wody w temperaturze pokojowej przez 16 godzin) i gotowano w garnku z przykrywką (100 ± 2 g w 400 ml, całkowity czas gotowania 25 min);
***** washed, peeled and grinded were boiled in covered saucepans (100 ±2 g in 250 ml water, 10 min total cooking time – obrane i pokrojone gotowano w garnku z przykrywką (100 ±2 g w 250 ml, 10 min całkowity czas gotowania).
Source – Źródło: Bassett and Sammán 2010, Konnings et al. 2001.

Loss in folates during heat treatment depends not only on acidity and the level of antioxidants, but also on the content of oxygen and metals. It should be emphasized that in products containing thermally stable lipophilic antioxidants (vitamin A, E, D i β-caroten) folate losses are smaller than in products with a lower content of the abovementioned vitamins. In the case of onions, beans and chicken eggs folate loss during thermal processing is approx. 10%. However, after cooking broccoli and lentil (10 and 25 min respectively) there is only 47% and 43% folate left (Table 3). In contrast, in frozen products folate losses are much greater. This is due to poor stability of folates during storage in frozen vegetables. The freezing process itself results in reducing the folate content of 10–20%, and after 3 months of storage the loss in various vegetables increases by 16 up to almost 100% (Table 4).

Table 4. The influence of freezing and freezer storage on folates content in food
Tabela 4. Wpływ mrożenia i przechowywania zamrażalniczego na zawartość folianów w żywności

| Content 5CH3-TH4-folates (μg · 100 g$^{-1}$ fresh vegetables) Zawartość 5CH3-TH4-folianów (μg · 100 g$^{-1}$ świeżych warzyw) | | Reduction [%] Stopień redukcji [%] – Freezing storage Przechowywanie zamrażalnicze | | | |
|---|---|---|---|---|---|
| | | after freezing po zamrożeniu | after 3 months po 3 miesiącach | after 9 months po 9 miesiącach | after 12 months po 12 miesiącach |
| Peas Groszek | 75.6 | 20.2 | 39.5 | 96.2 | 96.1 |
| Yellow beans Fasola żółta | 41.1 | 1.0 | 0.6 | 95.5 | 97.9 |
| Green beans Fasola zielona | 70.1 | 10.9 | 16.3 | 75.4 | 90.4 |
| Cauliflower Kalafior | 89.3 | 8.7 | 97.8 | 98.8 | 98.8 |
| Broccoli Brokuły | 158.5 | 9.7 | 23.6 | 99.0 | 99.4 |
| Spinach Szpinak | 180.2 | 26.2 | 39.1 | 90.5 | 91.9 |

Source – Źródło: Czarnowska and Gujska 2012.

## Folate bioavailability

The bioavailability of folates received from a diet, that is absorbed and metabolic utilization, lies between 30% to 98% (Brouwer et al. 1999, Hannon-Fletcher et al. 2004). Folates supplied with a diet mostly come in the form of reduced polyglutamates and in order to have been absorbed by the intestinal mucosa must be deconjugated to monoglutamates with the participation of glutamate carboxypeptidase (deconjugation of folates). Carboxypeptidase activity depends on pH of the environment (highest in the range of 6.5–7) and the presence of conjugation inhibitors (including fiber, organic acids) (Laskowska-Klita et al. 2012, Bassett and Sammán 2010). In vegetables such as cabbage, tomatoes, beans there are natural conjugation inhibitors in the form of Lower molecular mass organic acids (e.g. citrate, malonate). Their presence is also found in oranges, therefore bioavailability of polyglutamine folates from orange juice is approx. 67%. Foliate deconjugation activity is also inhibited by bacteriostatic medicines, non-steroidal anti-inflammatory drugs, oral contraceptives, alcohol abuse and smoking; this results in reduction of bioavailability of folates (Czeczot 2008). Bioavailability of folates coming from food is limited because they are bound by different macromolecules using covalent bonds. For example, polyphenols present in wine, beer and tea reduce the intake of folic acid by Caco-2 cells (Lemos et al. 2007). Limited bioavailability of folate is also connected with the possibility of degradation of unstable tetrahydrofolate in the acidic environment of the stomach. During *in vitro* digestion of milk significant losses in 5-methyl-TH4-folate were noted 10–60%. The addition of 0.01% of sodium ascorbate significantly increased retention of folate (to 90%) (Wigertz 1997). The absorption of folates takes place in the jejunum. Passive diffusion is possible only at certain concentrations of folate, amounting to more than 5 μM (Halsted 1990). At low concentrations (to 2 μmol · 1 L$^{-1}$) folates are absorbed through active transport, after prior combination with RFCs protein which binds only reduced folates, or with FBP that binds both reduced and oxidized forms of folates. The ability to bind folates with carrier proteins depends on pH and is the highest in acidic environment. Increase in the activity of carrier proteins is affected by the presence of sphingolipids and cholesterol, while substances such as mycotoxins, statins and also alcohol abuse limit the absorption of folate (Forssén et al. 2000, Laskowska-Klita et al. 2012). It should be emphasized that the FBP protects folates against degradation, thus increasing their stability and absorption in the small intestine and prevents the use of folate by the gut microflora (Eitenmiller and Landen 1999, Jones and Nixon 2002). Intestinal absorption of free and protein-bound folates takes place in two different parts of the small intestine. Monoglutamate forms of folates are absorbed in the jejunum, whereas the protein-bound folates are absorbed primarily in the ileum and their absorption is much slower. Slower rate of absorption of protein-bound folates and protection against intestinal bacteria increases their bioavailability. This is confirmed by the fact that breast-fed children excreted 55 μg of folate daily, while infants fed with milk replacers, in which the folate binding proteins are not present, excreted 78 μg (Ek and Magnus 1979, Said et al.1986). In studies conducted by Verwei et al. (2003) it was found that the absorption of folic acid from fortified milk without the addition of FBP was lower than the absorption of 5-methylo-TH4-folate. Adding FBP to milk reduced the absorption of folic acid even more, but had no effect on the absorption of 5-methylo-TH4-folate (72%) (Verwei et al. 2003).

## CONCLUSIONS

Due to low intake of fresh, biologically active food 90% of Polish women and 78% of men suffer from folic acid deficiency (Waśkiewicz et al. 2010). In long-term stored vegetables, frozen, for instance, especially in products with a high degree of processing, the folate contents is much lower because of their low stability. Animal products (eggs, fermented dairy products, ripened cheeses, meat, poultry, salmon) are an underestimated source of folate. Folate content in these foods is lower than in vegetables. However, due to the presence of thermostable lipophilic and hydrophilic antioxidants the folates present in animal products feature high stability and greater bioavailability. Consumption of fresh, biologically active food do not always cover the need for folate, especially in women during pregnancy (due to the prevention of neural tube defects), as well as during lactation. Furthermore, folic acid deficiency often occurs in the elderly. Because of folate's significant role in the prevention of atherosclerosis, diseases of the nervous system and colon cancer, supplementation, as well as folic acid food fortification is more than justified.

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**Abstract.** Food of plant origin is characterized by the highest content of folates. Unfortunately, these compounds are supple to oxidation; their thermal destruction and removing with water are also unavoidable. Fresh food products subjected to thermal processing contain only from about 40% (broccoli, lentils) to over 90% (onion, soya, hen's egg) of the initial amount of folates. Content of folates in chilled food subjected to thermal processing is much lower: loss during chilling amounts to 10–20%, and increase from 16 to almost 100% during storage. Moreover, inhibitors of conjugase present in vegetables (cabbage, tomatoes, beans) and oranges and also polyphenols (wine, beer, tea, fruits) reduce bioavailability of folates. In food of animal origin

(except liver) folate content is on average 2–2.5 times lower then in vegetables. Anyway, they are much more stable due to the presence of lipophylic antioxidants and also FBP (folate binding protein) in dairy products. Slower rate of absorption of folates bound to the protein and protection against intestinal bacteria increases their bioavailability. Absorption and metabolic utilization of folates is higher in the case of animal food products then plant ones.