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VITAMIN B_2 (RIBOFLAVIN) CONTENT IN CEREAL PRODUCTS

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Abstract

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Vitamin B_2 (riboflavin) is a water-soluble essential vitamin. Nowadays an increased risk for riboflavin deficiency may be seen in people on special diets (*diabetes mellitus*), smokers or heavy alcohol drinkers. In the Czech diet the main sources of the vitamin intake are milk and dairy products followed by cereals and meat. Cereals are good source of this vitamin as it is widely and regularly consumed in different forms. Analyses of the vitamin B_2 content in different types of cereal products (flours, breads, pastries, breakfast cereals, cooked pasta) of Czech origin using HPLC with reversed phase and UV detection were done. The vitamin B_2 content of chosen cereal products decreased in this progression: enriched wholemeal breakfast cereals (the best source of the vitamin), enriched wheat flours, breads – rye and wholemeal wheat breads, whole wheat and spelt flours, wheat bread, cooked whole wheat and rye spaghetti, wheat and multigrain pastries and finally scoured wheat flours.

vitamin B_2, riboflavin, cereal products, HPLC/UV

Vitamin B_2 – riboflavin is a water-soluble essential vitamin which is needed in the metabolism of amino acids, fatty acids, and saccharides. It also activates vitamin B_6 (pyridoxine) and folic acid and helps to create niacin. Riboflavin exerts its biological function through two coenzymes – flavine adenine dinucleotide (FAD) and flavine mononucleotide (FMN) which participate in oxidation-reduction reactions in numerous metabolic pathways exemplified by the oxidation of reduced NADH, the oxidation in the tricarboxylic acid cycle, and in the β–oxidation of fatty acids (Ball, 2006).

Humans cannot synthesize this vitamin and thus it must be obtained from their diet through absorption in the small intestine. The recommended daily intake (RDI) of riboflavin, related to protein and energetic intake, is from 1.2 to 1.7 mg per day in the Czech Republic (Velíšek, 2009).

Nowadays an increased risk for riboflavin deficiency may be seen in people on special diets (*diabetes mellitus*, peptic ulcer disease, weight loss), smokers, heavy alcohol drinkers and women using certain types of birth control. Therefore the solution could be the diet enriched with some of common vitamin sources as products with whole cereals or riboflavin enriched products, e.g. bread and pastries. These products could be enriched by riboflavin (E 101) up to 50% of RDI, cereal mixtures and pasta even up to 100% of RDI. Vitamin fortification of foods is referred in Regulation No. 446/2004. This vitamin can be also used as a colorant which is referred in Regulation No. 304/2004.

Great natural food sources of vitamin B_2 include: entrails – liver, nuts, yeast, cheese, eggs, milk, lean meat and wholemeal cereals. In the Czech diet the main sources of the vitamin intake are milk and dairy products followed by cereals and meat (Hlúbik and Opltová, 2004). Cereals are good source of this vitamin as it is widely and regularly consumed in different forms – flour for home culinary purposes and technological production of cereal products, bread, pastries, breakfast cereals, pastas, and biscuits, especially for common cereals – wheat and rye.

The aim of this work was therefore to determine riboflavin content in common cereal products of the Czech origin.

Riboflavin is stable, not destroyed by heat or oxidation, unless exposed to ultraviolet radiation (420–560 nm). So it is a highly photosensitive compound. Knowledge of its photochemical behaviour in aqueous solution over an appropriate pH range is needed to predict the shelf-life. The choice of the optimum pH is crucial for liquid preparations. Ahmad *et al.* (2004) determined that riboflavin solutions are most stable to UV and visible radiations at pH 5–6. Riboflavin content could be also decreased according to character of foods, technological and culinary processes (Lešková *et al.*, 2006), e.g. scouring and dissolution in cooking water (Al-Khalifa and Dawood, 1993).

Vitamins (vitamin B_2) in cereals occur especially in germ and aleuronic layer, so losses are connected to cereal scouring, primarily its level (Hägg and Kumpulainen, 1994). Scoured cereal products without bran then contain up to ten times less B-complex vitamins than bran products. Losses of riboflavin due to baking are quite small (up to 10%). The significant losses of this vitamin (35–55%) are in cooked products due to leaching into water (Ayranci and Kaya, 1993; Velíšek, 2009). Freezing doesn't significantly affect riboflavin contents in breads (Hägg and Kumpulainen, 1994).

In foodstuffs, the vitamin B_2 may be presented in free and phosphorylated forms, tightly bound but non-covalently to proteins. For vitamin extraction there can be used an acid hydrolysis or an enzymatic treatment (*takadiastasa, trypsin, clarasa*) (Ndaw *et al.*, 2000).

For vitamin determination several methods have been reported – microbiological assay (Golbach *et al.*, 2007), electrochemical method and High Performance Liquid Chromatography (Arella *et al.*, 1996, Tang *et al.*, 2006). The most widely used method in the determination of vitamin B_2 is HPLC using reverse phase with fluorescence (Andrés-Lacueva *et al.*, 1998; Cataldi *et al.*, 2003) and UV (Hőller *et al.*, 2003) detection.

MATERIAL AND METHODS

Samples

20 cereal products are from the Czech production purchased in food markets.

- flours – wheat (bread, standard, pastry) flours
  - whole wheat and spelt flours
  - enriched wheat (bread, standard, pastry) flours – declared amount of riboflavin content of all enriched flours: 0.640 mg.100 g^{-1}
- breads: wheat, wholemeal (2 types), rye bread
- breakfast cereals: enriched wholemeal (3 types)
- pastries: wheat, multigrain (2 types) pastries
- cooked spaghetti: whole wheat and rye spaghetti (cooked in water at 90 °C for 10 min).

Reagents

- Calibration: riboflavin was obtained from Supelco (USA)
  - standard solutions: 0.25–4 µg.ml^{-1}
- extraction: HCl, trichloroacetic acid, Carrez I and Carrez II were obtained from P. Lukeš (Czech Republic)
- HPLC determination: CH_3COONa was purchased from P. Lukeš (Czech Republic), methanol of HPLC grade from Sigma – Aldrich Chemicals (Germany).

Vitamin extraction

- 100 ml 0.2 M HCl and 3 ml CCl_3COOH
- 30 ml of Carrez I and Carrez II.

Chromatographic conditions

- HPLC (Hewlett Packard 1100, USA)
- injection volume 20 µl
- mobile phase: gradient technique.

$$CH_3COONa : CH_3OH\ 87:13$$

- column SUPELCOSIL – LC8 (15 cm × 4.6 mm; 5 µm, Supelco, USA)
- column temperature 30 °C
- flow rate: 0.8 ml.min^{-1}
- Detector: UV/VIS DAD G1315A

$$\lambda = 270\,\text{nm}.$$

Riboflavin Extraction and HPLC Determination

By reason of complicated matrices based on protein-polysaccharides, the first step was isolation of vitamin B_2 by acid hydrolysis. That is essential to denature the proteins and to release the vitamin from his association with the proteins and when the foodstuffs contain a lot of starch, to convert it into soluble form (Ndaw *et al.*, 2000).

Extraction was done with 0.2 M hydrochloric acid in a water bath at 95 °C for 1 hour with addition of 80% trichloroacetic acid in 50 minute. Moreover the proteins were subsequently precipitated by solutions Carez I and Carez II followed by filtration. All samples were kept away from light because of its low photostability.

The suitable conditions for the determination by HPLC technique were established partly according to Buňka *et al.* (2003). Mobile phase composed of sodium acetate, pH adjusted to 4.5 as riboflavin solutions are most stable to UV and visible radiations at pH about 5–6, and methanol, in the ratio 87:13 (v/v) with gradient technique (till 15 min., then 0:100) for 30 min. and flow rate 0.8 ml.min^{-1}.

For riboflavin detection UV/VIS DAD detector was used at the wavelength of 270 nm with ChemStation-Instrument 1 programme for the content calculation.

Method validation

Confirmatory analysis of validation parameters were experimentally verified and confirmed, as previously published by Buňka *et al.* (2003).

Calibration curve (y = ax + b): y = 79.572x – 2.243

Correlation coefficient: r = 0.9995

Recovery: 90.1 ± 2.3 % – 91.4 ± 3.1 % (known amount of vitamin B_2 was added to cereal samples)

Inter and intra-day repeatability: SD < 3 %.

RESULTS AND DISCUSSION

Table I. presents the content of riboflavin in 20 cereal products – flours, breads, pastries, breakfast cereals and cooked spaghetti. There is also shown contribution of 100 g cereal product to RDI (Recommended Daily Intake in the Czech Republic is 1.5 mg per day).

The vitamin B_2 content of chosen Czech cereal products decreased in this progression: enriched wholemeal breakfast cereals, enriched wheat flours, breads – rye and wholemeal wheat breads, whole wheat and spelt flours, wheat bread, cooked whole wheat and rye spaghetti, wheat and multigrain pastries and finally scoured wheat flours.

From the chosen products the best ones as for riboflavin content were wholemeal breakfast cereals (0.553–2.779 mg.100 g^{-1}) enriched with vitamins. This range represents 36.9–185.3 % contribution to RDI for riboflavin. The results are in agreement with the literature (Hägg and Kumpulainen, 1993). Bran cereals also contain more vitamin B_2 than scoured ones as it was previously referred (Hägg and Kumpulainen, 1994). These products don't need any further culinary treatment so they are really good source of this vitamin.

Also all enriched wheat flours – bread (Fig. 1A), pastry and standard (0.525–0.800 mg.100 g^{-1}; contribution of 35.0–53.3 % to RDI) and whole spelt and wheat flours (0.204 and 0.265 mg.100 g^{-1}) seem as good sources. The vitamin concentration in scoured flours was very low (up to 0.087 mg.100 g^{-1} in wheat bread flour (Fig. 1B)), as expected. The results correspond to the producer values and are also in agreement with the literature (Batifoulier *et al.*, 2005; Hägg and Kumpulainen, 1994; Ndaw *et al.*, 2000). The variability of the content in flours could be explained by milling procedure, genetic variability and growing location. So milling and the type of the flour (bread, standard, pastry) are very important for the responsibility in the differences of the vitamin results. And flours before consumption need another technological or culinary treatment so it could lead to another loss of vitamin due to that preparation.

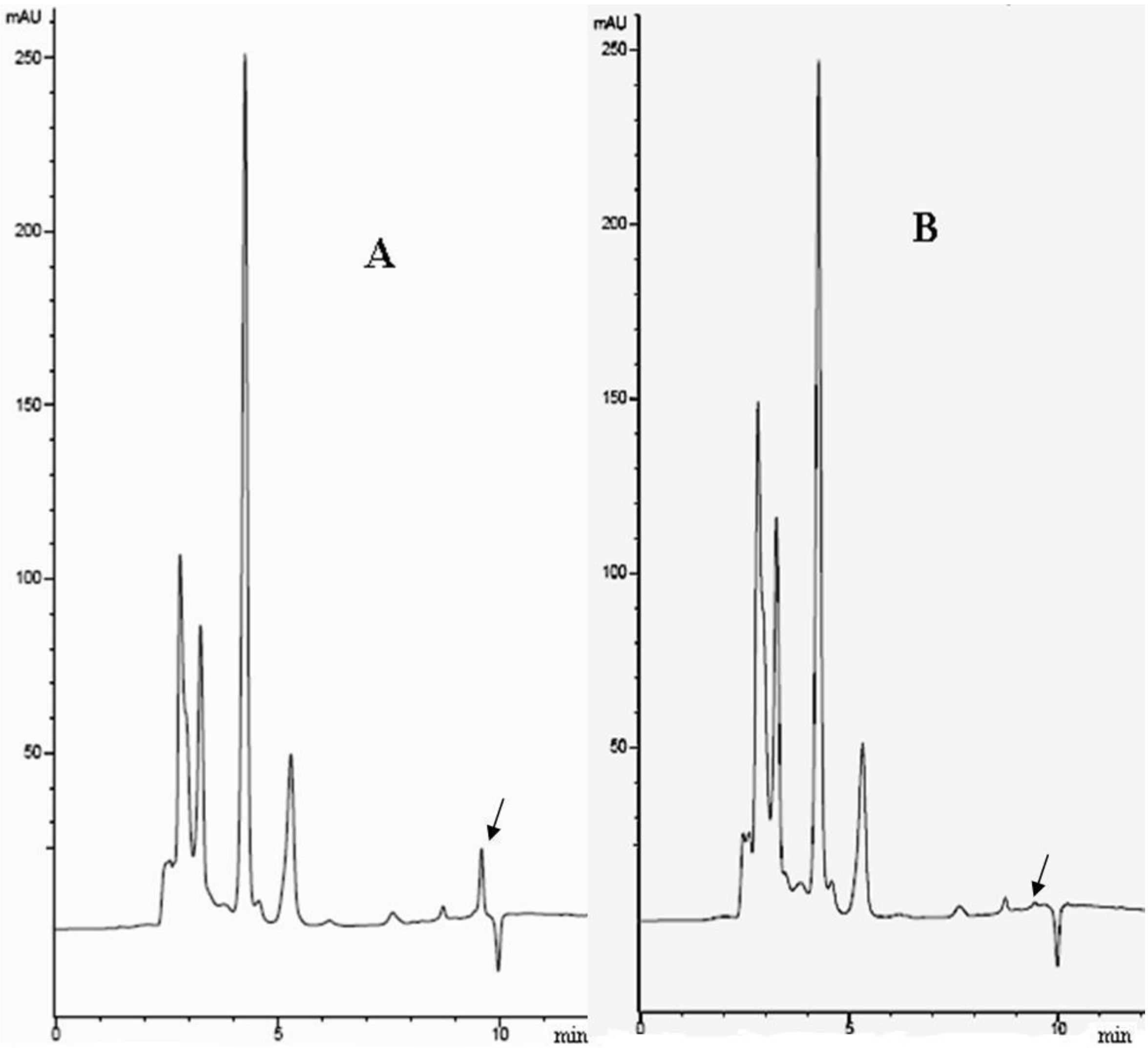
Riboflavin content in bread products was higher than in flours. The concentration increased during bread making, suggesting a potential synthesis or contribution by yeast as referred Batifoulier *et al.* (2005) and Batifoulier *et al.* (2006).

Lower vitamin concentration was found in common wheat bread (0.208 mg.100 g^{-1}) than in wholemeal and rye breads (0.322–0.561 mg.100 g^{-1}; contribution of 21.5–37.4% to RDI). This could be related to the flour scouring level as the vitamin is contained especially in the bran. It reflects a more dras-

I: *Riboflavin content in cereal products (meanSE) [mg.100 g^{-1}] and contribution of 100 g product to RDI [%]*

Cereal products	Average riboflavin content	Contribution of 100 g product to RDI
Flour: Enriched wheat bread	0.800 ± 0.004	53.3
Enriched wheat pastry	0.584 ± 0.006	38.9
Enriched wheat standard	0.525 ± 0.005	35.0
Whole wheat	0.265 ± 0.005	17.7
Whole spelt	0.204 ± 0.004	13.6
Wheat bread	0.087 ± 0.006	5.8
Wheat pastry and standard	nd.	-
Bread: Rye	0.561 ± 0.010	37.4
Wholemeal 1	0.504 ± 0.013	33.6
Wholemeal 2	0.322 ± 0.013	21.5
Wheat	0.208 ± 0.008	13.9
Pastries: Wheat	0.094 ± 0.007	6.3
Multigrain 1	0.081 ± 0.007	5.4
Multigrain 2	nd.	-
Wholemeal breakfast cereals: 1	2.779 ± 0.007	185.3
2	1.246 ± 0.010	83.1
3	0.553 ± 0.012	36.9
Cooked spaghetti: Whole wheat	0.179 ± 0.007	11.9
Whole rye	0.223 ± 0.010	14.9

nd. – not detected



1: *Chromatographic analysis of vitamin B_2 in enriched wheat bread flour (A) and wheat bread flour (B) by HPLC/UV*

tic milling effect with refined flour than with unrefined, whole flour (Hägg and Kumpulainen, 1994).

Low concentrations of riboflavin in cooked spaghetti (0.179–0.223 mg.100 g^{-1}) of Czech origin, though wholemeal product, are due to vitamin leaching into water which correspond to results of Ayranci and Kaya (1993). According to them the percentage of riboflavin loss for macaroni during cooking at 90 °C was 18.3 %, (10 min cooking with water) and 53.3 % (20 min cooking).As expected, the lowest vitamin B_2 levels were found in pastries (up to 0.094 mg.100 g^{-1}) made from scoured flours. So, common wheat or multigrain pastries are not very good source of this vitamin in the Czech Republic.

Riboflavin is water-soluble vitamin which leaches into water, e.g. during cooking with water. Other losses could happen during storage e.g. UV radiation impact. In some papers there have been proved that riboflavin losses depend on technological condition (Batifoulier *et al*., 2005; Batifoulier *et al*., 2006; Hägg and Kumpulainen, 1994; Prodanov *et al*., 2004).

SUMMARY

Riboflavin is a water-soluble essential vitamin which is needed in the metabolism of amino acids, fatty acids, and saccharides (RDI=1.5 mg per day). Nowadays an increased risk for riboflavin deficiency may be seen in people on special diets (e.g. *diabetes mellitus*). In the Czech diet the main sources of the vitamin intake are milk and dairy products followed by cereals and meat. Cereals are good source of this vitamin as it is widely and regularly consumed in different forms. The aim of this work was therefore to determine riboflavin content in common cereal products of the Czech origin.

One of widely used method in the determination of vitamin B_2 is HPLC using reverse phase with UV detection. The vitamin B_2 content of chosen Czech cereal products, determined by this method, decreased in this progression: enriched wholemeal breakfast cereals (0.553–2.779 mg.100 g^{-1}; contribution of 36.9–185.3 % to RDI), which are really good source of the vitamin as they don't need any further culinary treatment; then enriched flours (0.525–0.800 mg.100 g^{-1}), breads – wholemeal with higher content as vitamin (0,322–0,561 mg.100 g^{-1}) which occurs especially in grain germ and aleuronic layer; following by wheat bread (0.208 mg.100 g^{-1}). In breads, in comparison to flours, could the riboflavin content increased during bread making (synthesis or by yeast contribution); next wheat flours – due to the cereal scouring the concentration was very low (up to 0.087 mg.100 g^{-1}; contribution of 5.8 % to RDI). Low concentrations of riboflavin in cooked spaghetti (0.179–0.223 mg.100 g^{-1}, contribution of 11.9–14.9 % to RDI) are due to vitamin leaching into water. The lowest vitamin B_2 levels were found in pastries (up to 0.094 mg.100 g^{-1}), so common wheat or multigrain pastries from scoured flours are not very good source of this vitamin.

SOUHRN

Obsah vitaminu B_2 (riboflavinu) v cereálních produktech

Riboflavin je ve vodě rozpustný vitamin, který je zapotřebí pro metabolismus aminokyselin, tuků a sacharidů (DDD = 1,5 mg na den). V dnešní době mají někteří lidé (např. nemocní s *diabetes mellitus*) větší potřebu přísunu vitaminu B_2. Zdroje tohoto vitaminu u lidí v České republice pochází především z mléka a mléčných výrobků, cereálií a masa. K nejvýznamnějším rostlinným zdrojům těchto vitaminů patří obiloviny kvůli jejich běžné a poměrně rozsáhlé konzumaci v různé formě. Cílem této práce proto bylo stanovení obsahu riboflavinu v běžných cereálních výrobcích českého původu.
Jednou z nejvíce rozšířených metod pro jeho stanovení je HPLC s reverzní fází a UV detekcí. Obsah vitaminu B_2 ve vybraných cereálních produktech, stanovený výše uvedenou metodou, klesal v tomto pořadí: obohacené celozrnné snídaňové cereálie (0,553–2,779 mg.100 g^{-1}; kde 100 g výrobku zabezpečí 36,9–185,3 % DDD riboflavinu), které jsou tedy nejlepším zdrojem z hodnocených produktů bez nutnosti dalších kulinárních úprav. Následují vitaminem obohacené mouky (0,525–0,800 mg.100 g^{-1}), dále chleba – celozrnný s vyšším obsahem vitaminu (0,322–0,561 mg.100 g^{-1}), který se nachází především v klíčku a aleuronové vrstvě, a pšeničný chleba (0,208 mg.100 g^{-1}). Chleba v porovnání s odpovídajícími moukami může mít vyšší obsah vitaminu B_2 z důvodu nárůstu v průběhu pečení (syntéza nebo přídavek droždí). Bílé pšeničné mouky měly poměrně nízký obsah riboflavinu s ohledem na vymílání těchto mouk (do 0,087 mg.100 g^{-1}; 5,8 % DDD). Nízké obsahy riboflavinu ve vařených špagetách (0,179–0,223 mg.100 g^{-1}, 11,9–14,9 % DDD) souvisí s vyluhováním do vodného prostředí. Nejnižší hodnoty obsahů vitaminu B_2 byly zjištěny v pečivu (do 0,094 mg.100 g^{-1}), tedy běžné vymílané bílé a vícezrnné pečivo není výhodným zdrojem tohoto vitaminu.

vitamin B_2, riboflavin, cereální produkty, HPLC/UV

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