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CHROMATOGRAFICKÉ STANOVENIE RIBOFLAVÍNU (VITAMÍN B$_2$) V CELOZRNNÝCH CEREÁLNYCH VÝROBKOCH
CHROMATOGRAPHIC DETERMINATION OF RIBOFLAVIN (VITAMIN B$_2$) IN WHOLEMEAL CEREAL PRODUCTS

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Abstract: Riboflavin (vitamin B$_2$) is essential for humans due to its function in metabolism of amino acids, saccharides and fatty acids. To the most considerable sources of riboflavin intake in the Czech diet belong milk and dairy products, meat and cereals. Wholemeal cereals and their products are considered to be the better sources of this vitamin than scoured cereal products. Determination of the vitamin content in different wholemeal cereal products (flours, breads, pastries, biscuits, pastas) of Czech origin was done by HPLC with UV detection. Riboflavin content of chosen whole grain cereal products decreased in this progression: wholemeal biscuits; whole grain breads; whole wheat, rye and spelt flours; whole wheat and graham pastas and finally different types of wholemeal pastries.
Keywords: vitamin B$_2$, riboflavin, determination, HPLC/UV, wholemeal cereals

INTRODUCTION

Riboflavin - vitamin B$_2$ is essential water-soluble vitamin. It is needed in the metabolism of amino acids, saccharides and fatty acids through two coenzymes – FAD and FMN. It also activates vitamin B$_6$ and folic acid and helps to create niacin. This vitamin must humans obtain from the diet. Good natural food sources of riboflavin include: entrails – liver, yeast, nuts, milk and dairy products such as cheese, eggs, lean meat and 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 generally good source of this vitamin as it is widely and regularly consumed in different forms (cereal products such as bread, pastries, breakfast cereals, pasta). Common cereal products are produced especially from wheat and rye. Whole grain cereals and their products are considered to be the better sources of this vitamin than scoured cereal products due to its higher content (**Batifoulier et al., 2005**).

The recommended daily intake (RDI) of riboflavin is related to protein and energetic intake. In the Czech Republic the RDI value is enacted to 1.4 mg per day (**Regulation No. 352/2009**).

Some cereal products, especially scouted ones, 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.

Riboflavin is stable, unless exposed to ultraviolet radiation (420–560 nm); most stable to UV and visible radiations at pH 5–6 **(Ahmad et al., 2004)**. Riboflavin content could be decreased during technological and culinary processes (**Lešková et al., 2006**), such as scouring, because vitamins in cereals occur especially in germ and aleuronic layer. Scoured cereal products without bran then contain up to ten times less B-complex vitamins than bran products (**Hägg and Kumpulainen, 1994**).

Vitamin B$_2$ occurs in free and phosphorylated forms in foods, bound to proteins. For vitamin extraction an acid hydrolysis (HCl) or an enzymatic treatment (*trypsin, takadiastasa*, *clarasa*) are used (**Ndaw et al., 2000**). For riboflavin determination several methods have been reported – microbiological assay, electrochemical method and HPLC technique (**Arella et al., 1996**). The most frequent method is HPLC using fluorescence and UV detection (**Anyakora et al., 2008**).The aim of this work is to determine riboflavin content by HPLC/UV in whole grain cereal products of the Czech origin.

MATERIALS AND METHODS

Samples: 20 whole grain cereal products from the Czech production purchased in food markets – flours (4 types), breads (3 types) and pastries (6 types), biscuits (4 types) and pastas (3 types).

Reagents: extraction - HCl, trichloroacetic acid, Carrez I and Carrez II (P. Lukeš, Czech Republic); riboflavin (Supelco, USA) for standard solutions: 0.25 - 4 $\mu g.ml^{-1}$; HPLC determination: CH_3COONa (P. Lukeš, Czech Republic), methanol of HPLC grade (Sigma Aldrich Chemicals, Germany).

Vitamin extraction: 100 ml 0.2 M HCl, water bath at 95 °C for 1 hour, 3 ml 80 % CCl_3COOH, followed by decantation with Carrez I and Carrez II.

Chromatographic conditions of HPLC (Hewlett Packard 1100, USA): injection volume 20 µl; mobile phase (pH=4.5): CH_3COONa : CH_3OH, gradient technique 87:13 (v/v) till 15 min., then 0:100 till 30 min; column SUPELCOSIL - LC8 (15 cm x 4.6 mm; 5 µm; Supelco, USA); column temperature 30 °C; flow rate: 0.8 $ml.min^{-1}$; detector: UV/VIS DAD G1315A, λ=270 nm.

Method validation: Calibration curve: y = 78.742 . x + 0.535; correlation coefficient: r = 0.9989; recovery: 91.2 ± 3.1 % – 91.7 ± 3.4 % (known amount of vitamin B_2 was added to cereal samples); inter and intra-day repeatability: SD < 3 %.

RESULTS AND DISCUSSION

Table 1 presents the content of riboflavin determined by HPLC/UV in 20 wholemeal cereal products – flours, breads, pastries, biscuits and pastas. There is also shown contribution of 100 g wholemeal cereal product to RDI (1.4 mg per day).

The vitamin B_2 content of chosen Czech whole grain cereal products decreased in this progression: wholemeal biscuits; whole grain breads; whole wheat, rye and spelt flours; cooked whole wheat and graham pasta and finally wholemeal pastries.

Bran cereal products generally contain higher content of vitamin B_2 than scoured ones as previously referred **(Hägg and Kumpulainen, 1994**, **Hägg and Kumpulainen, 1993**, **Škrovánková and Sikorová, 2010)** with wholemeal breakfast cereals, enriched with vitamins, as the best source of riboflavin.

From the chosen Czech products the best ones, as for riboflavin content, were wholemeal biscuits (Fig. 1) (0.496 – 0.522 $mg.100\ g^{-1}$), the range represents 35.4 – 37.3 % contribution to RDI for riboflavin.

Also whole grain breads (0.307 – 0.510 $mg.100\ g^{-1}$) seem as good sources. Losses of riboflavin due to baking are quite small (up to 10 %) (**Hägg and Kumpulainen, 1994).** Riboflavin content in bread products was higher than in flours (whole wheat, rye and spelt) (0.180 – 0.255 $mg.100\ g^{-1}$). The vitamin concentration increases presumably during bread making, suggesting a potential synthesis or contribution by yeast (**Batifoulier et al., 2005, Batifoulier et al., 2006).** The variability of the content in flours could be explained by milling procedure, genetic variability and growing location.

Lower vitamin concentration was found in cooked pastas, probably due to vitamin leaching into water e.g. during cooking with water. Low concentrations of riboflavin were found in pastries (up to 0.107 $mg.100\ g^{-1}$).

Tab. 1 Riboflavin content of whole grain cereal products (mean ± SE) [mg.100 g^{-1}] and contribution of 100 g product to RDI [%]

Whole grain cereal product	Riboflavin content	Contribution of 100 g product to RDI
Bio wholemeal biscuits	0.522 ± 0.005	37.3
Whole spelt biscuits	0.520 ± 0.003	37.1
Graham biscuits	0.505 ± 0.005	36.7
Whole wheat crunchy bread	0.510 ± 0.006	36.4
Wholemeal biscuits	0.496 ± 0.003	35.4
Whole wheat bread 1	0.437 ± 0.007	31.2
Whole wheat bread 2	0.307 ± 0.001	21.9
Whole wheat flour	0.255 ± 0.004	18.2
Whole rye flour	0.220 ± 0.001	15.7
Whole spelt pastry flour	0.191 ± 0.002	13.6
Graham pasta	0.185 ± 0.001	13.2
Whole wheat noodles	0.181 ± 0.002	12.9
Whole spelt bread flour	0.180 ± 0.002	12.9
Whole spelt long noodles	0.167 ± 0.002	11.9
Graham French loaf	0.107 ± 0.005	7.6
Graham roll 1	0.104 ± 0.005	7.4
Graham roll 2	0.101 ± 0.001	7.2
Graham roll 3	0.101 ± 0.002	7.2
Graham bun	0.101 ± 0.003	7.2
Graham roll 4	0.093 ± 0.004	6.6

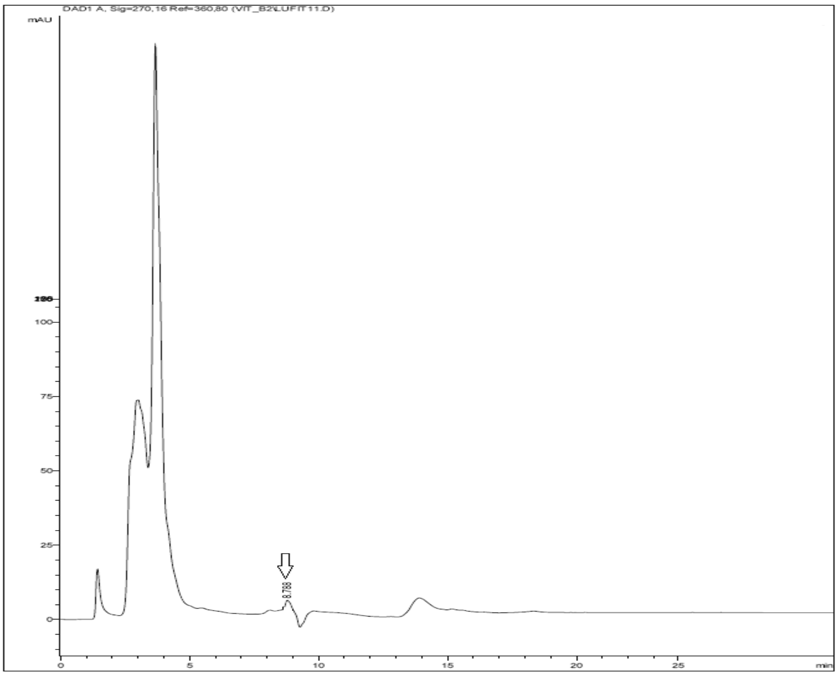

Fig. 1 Chromatogram of riboflavin by HPLC/UV in wholemeal biscuits

CONCLUSION

Wholemeal cereal products are good source of riboflavin (vitamin B_2) as they are widely and regularly consumed in different forms. The vitamin content is higher than in scoured products as vitamins in cereals occur especially in germ and aleuronic layer. The determination of vitamin B_2 was provided by HPLC/UV. The vitamin B_2 content of chosen Czech whole grain cereal products decreased in this progression: wholemeal biscuits (0.496 – 0.522 mg.100 g^{-1}; 35.4 – 37.3 % to RDI); whole grain breads (the content could be increased during bread making); whole wheat, rye and spelt flours; lower content in cooked whole wheat and graham pasta (due to vitamin leaching into water) and the lowest content was determined in different types of wholemeal pastries.

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