Folate quantification in food – pure plant deconjugase and LC-MS/MS for a future standard method

Ložnjak, Petra; Jakobsen, Jette

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Folate quantification in food

– pure plant deconjugase and LC-MS/MS for a future standard method

Petra Ložnjak*, Jette Jakobsen
National Food Institute, Technical University of Denmark, Denmark
*petlon@food.dtu.dk

Introduction

Folate or vitamin B_9 is a generic term for a group of water-soluble compounds that play a key role in one carbon transfer reactions in all living organisms. Non-specific microbiological assay using animal-origin ɣ-glutamyl hydrolase (GGH) is currently the standard method for folate quantification. However, there are discrepancies in the data for folate content between methods due to the lack of a proper deconjugase enzyme which would enable fast and reproducible folate deglutamylation. Within the framework of the Nordic Standardization Committee (NMKL), a collaborative study was conducted for a method capable to quantify the specific folate vitamers in food[1].

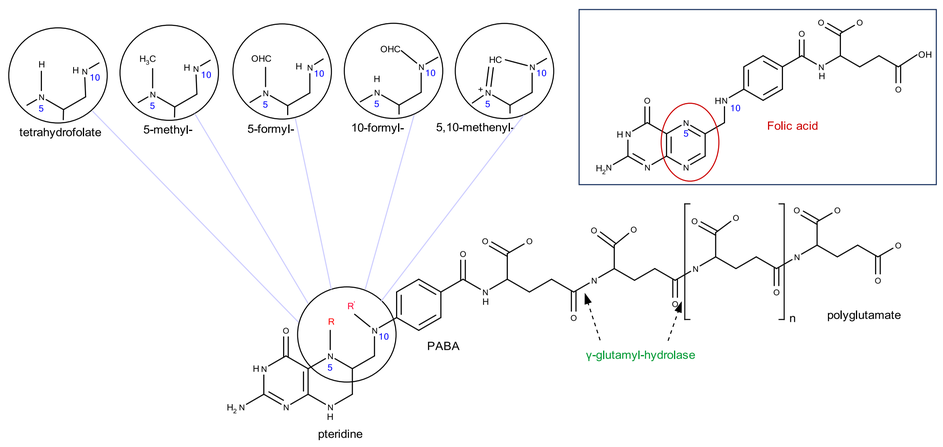


Figure 1 The structure of naturally-occurring folates and the role of ɣ-glutamyl hydrolase in analysis of folate. PABA: *para*-aminobenzoic acid

Method

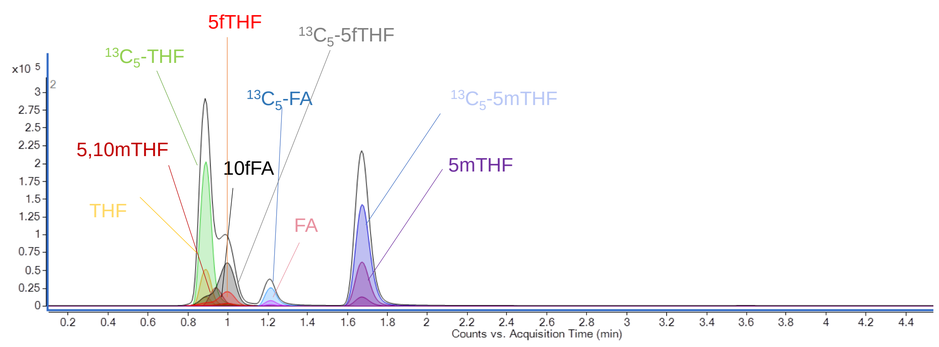
The newly developed method included single-enzymatic extraction step in which an enzyme of a plant origin (*Arabidopsis thaliana*) was used[1]. Six folate vitamers such as; tetrahydrofolate (THF), 5,10-methenyl-tetrahydrofolate (5,10mTHF), 10-formyl-folic acid (10fFA), 5-formyl-tetrahydrofolate (5fTHF), folic acid (FA) and 5-methyl-tetrahydrofolate (5mTHF) were quantified using the $^{13}C_5$-labeled internal standards by LC-MS/MS method. In a round robin study the method has earlier been compared to a microbiological assay and another LC-MS/MS method using animal-origin deconjugase resulting in a constant bias of -17% and 25%, respectively[2], which served as a basis for collaborative study.

40 g sample

Freeze with liquid N_2

Grind for 20 s

0.5 g of sample



Homogenization step as a part of the collaborative study

Collaborative study

- **8** European laboratories
- **5** practice samples (3 non-homogenized + 2 homogenized)
- **7** test materials (fruits, vegetables, legumes, dairy products, offal, fish and infant formula)
- **6** folate standards + **4** internal standards
- **1** Pure plant-origin ɣ-glutamyl hydrolase

Results

Table 1. The **precision** of the method for total folate analysis in the various food matrices.

Sample No.	Sample	Received as a homogenized sample	Total No. of labs	Total No. of replicates	Overall mean of all data (grand mean)	RSD_r	RSD_R	HorRat
			P	Sum [*n*(L)]	µg/100 g	%	%	HorRat
1	Strawberry	No	7	14	74	4.2	10.0	0.32
2	Spinach	No	8	16	200	9.9	28.0	0.88
3	Yoghurt	Yes	8	16	17	14.9	21.8	0.69
4	Lentils	No	7	14	112	2.3	19.2	0.61
5	Liver, pork	Yes	8	16	490	6.0	22.2	0.70
6	Salmon	Yes	8	16	23	10.6	58.3	1.84
7	NIST SRM 1869 Infant/Adult Nutritional Formula	Yes	7	14	259	4.6	8.7	0.27

Table 2. The **accuracy** of the method for folic acid analysis in the NIST SRM 1869 infant formula adult nutritional in comparison with the reference value

Parameters	**Symbol**	**Units**	**NIST SRM 1869**
Total No. of laboratories	*P*		7
Total No. of replicates	n		14
Overall mean of all data (grand mean)	Mean	µg/100 g	225
Repeatability standard deviation	SD_r	µg/100 g	11.0
Reproducibility standard deviation	SD_R	µg/100 g	22.4
Repeatability relative standard deviation	RSD_r	%	4.9
Reproducibility relative standard deviation	RSD_R	%	10.0
HorRat value	HorRat		0.31
NIST reference value[3]	Mean	µg/100 g	224
NIST reference range[3], minimum = mean – (1 x SD); maximum = mean + (1 x SD)	Minimum - maximum	µg/100 g	215-233

HorRat
0.5 - 2.0

Conclusion

The samples for the collaborative study were selected to be representatives for all food samples. Homogenization step was successfully implemented as part of the method by the laboratories. Lower precision was observed in samples with low folate content, whereas the HorRat value was within the acceptable range for all samples. The included reference material showed acceptable results. The next step for the standardization procedure is to publish the results, and get the plant-origin GGH commercialized.

References:
1. Ložnjak, P., García-Salinas, C., Díaz de la Garza, R. I., Bysted, A., & Jakobsen, J. (2019). The use of a plant enzyme for rapid and sensitive analysis of naturally-occurring folates in food by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A*, *1594*, 34-44.
2. Ložnjak, P., Striegel, L., Díaz de la Garza, R. I., Rychlik, M., & Jakobsen, J. (2020). Quantification of folate in food using deconjugase of plant origin combined with LC-MS/MS: A method comparison of a large and diverse sample set. *Food Chemistry, 305*, 125450.
3. SRM 1869; *Folic acid*; National Institute of Standards and Technology; U.S. Department of Commerce: Gaithersburg, MD (16 August 2018)