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Folate quantification in food

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

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Introduction

Folate or vitamin B₉ 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 γ -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 food¹.

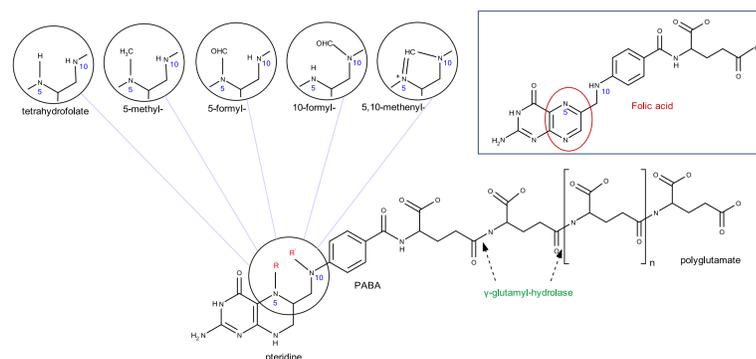
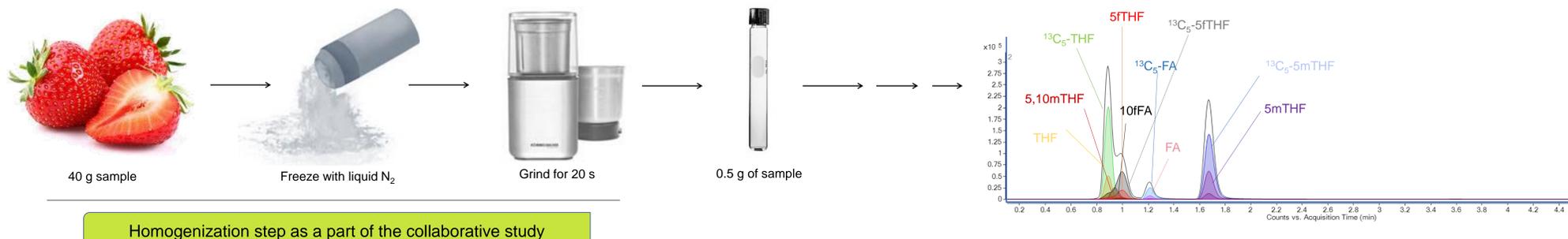


Figure 1 The structure of naturally-occurring folates and the role of γ -glutamyl hydrolase in analysis of folate. PABA: para-aminobenzoic acid

Method

The newly developed method included single-enzymatic extraction step in which an enzyme of a plant origin (*Arabidopsis thaliana*) was used¹. 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 ¹³C₅-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%, respectively², which served as a basis for collaborative study.



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, ofal, fish and infant formula)
- 6 folate standards + 4 internal standards
- 1 Pure plant-origin γ -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) $\mu\text{g}/100\text{ g}$ | RSD _r | RSD _R | HorRat |
|------------|--|----------------------------------|-------------------|-------------------------|--|------------------|------------------|--------|
| | | | P | Sum [n(L)] | | % | % | 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 | $\mu\text{g}/100\text{ g}$ | 225 |
| Repeatability standard deviation | SD _r | $\mu\text{g}/100\text{ g}$ | 11.0 |
| Reproducibility standard deviation | SD _R | $\mu\text{g}/100\text{ g}$ | 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 | $\mu\text{g}/100\text{ g}$ | 224 |
| NIST reference range ³ , minimum = mean - (1 x SD); maximum = mean + (1 x SD) | Minimum - maximum | $\mu\text{g}/100\text{ g}$ | 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.