Objective

- The goal of the study was to determine how long 5MTHF was stable in fresh, frozen, homogenized produce in order to validate the sample preparation and analysis protocol used for determination of folate in fresh produce sampled for the National Food and Health Analysis Program (NFNAP).

Samples and Sample Preparation

- Seven fruits and vegetables were chosen to give a broad representation of the types of produce analyzed in the NFNAP and were purchased locally (Blacksburg, VA). Other fresh produce was sampled according to a statistical probability prior to analysis in the laboratory. The local vegetable was chosen from available fresh market materials. The local vegetable was from a specific unit and not the same vegetable from a different unit.
- The local vegetable was stored at -60°C before the time of analysis.
- The local vegetable was stored at -60°C until the day of analysis.
- Samples were analyzed in triplicate immediately after homogenization, and then again after storage for 2, 7, and 30 days. Followed by analysis in triplicate approximately three months later to check for losses.

Analytical Method

Extraction From Sample Matrix

- Fruits were blended in a 3:1 ratio of sample matrix to extraction buffer in extraction buffer, followed by incubation at 37°C for 12 hours. The resulting extract was filtered through 0.45 µm filters before analysis.
- Samples were analyzed from the NFNAP within one year of the compositing date. All NFNAP composites were found to be stable during the period of analysis.
- Extraction from Sample Matrix was transferred to the appropriate sized volumetric flask, diluted to volume with extraction buffer and mixed.

Solid-Phase Extraction

- Spinach and homogenate extracts were cleaned up on a C18 Ag+/Phenyl SPE cartridge. The sample was quantitatively transferred to the appropriate sized volumetric flask, diluted to volume with extraction buffer and mixed.
- 1ml of the diluted extract was transferred to an amber HPLC autosampler vial.

Adjustment of Final Sample Concentration

- Fluorescence detector chromatograms were used for all quantitation.
- Using HPLC 50–80°C, the sample was quantitatively transferred to the appropriate sized volumetric flask, diluted to volume with extraction buffer and mixed.
- 1ml of the diluted extract was transferred to an amber HPLC autosampler vial.

Stability of 5MTHF

- The sample was incubated at 4°C in 50% water/50% methanol.
- The results validate the sample preparation and analysis protocol used for determination of folate in fresh fruits and vegetables in the National Foods Nutrient Analysis Program.

Method Validation and Quality Control

- Using HPLC 50–80°C, the sample was quantitatively transferred to the appropriate sized volumetric flask, diluted to volume with extraction buffer and mixed.
- 1ml of the diluted extract was transferred to an amber HPLC autosampler vial.

Conclusions

- This study was conducted as part of cooperative agreement #19-NW-11115-11 between the USDA Nutrient Data Laboratory and Virginia Polytechnic Institute and State University. The technical assistance of Ms. tasty and Mr. David Rigad in conducting sample analyses is acknowledged. The technical assistance of Ms. tasty and Mr. David Rigad in conducting sample analyses is acknowledged.
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References

- The specific methods and reagents used for determination of folate in fresh fruits and vegetables in the National Foods Nutrient Analysis Program are described in detail in the publication.
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