Quantitative Testing of Folic Acid in Fortified Corn Masa

To measure added folic acid (FA) in corn masa or corn masa flour, you can follow the methodology below, based on an article by Alaburda et al. (2007)[1] that validated and compared quantitative FA methods using HPLC and ELISA to measure added FA to wheat flour. The method detailed below is the HPLC method, which is similar to those historically used in industry. While the ELISA assay is simpler and provided similar results in this study, there is no report of long-term usage experience from industry for that assay. It appears that it may be an acceptable screening assay for processors with limited analytical testing facilities on-site.

An abbreviated summary of the reversed-phase HPLC method with ultraviolet detection at 280nm is provided below for use in fortified corn masa. Special thanks to Dr. Michael Dunn of Brigham Young University for his review of this document.

Required equipment and materials for HPLC:

- Standards/chemicals
  - Folic acid (FA) standard
  - Acetonitrile (ACN)
  - Acetic acid (AA)
  - Methanol
  - Sodium hydroxide
  - Dibasic sodium phosphate
  - Monobasic potassium phosphate
  - Sodium tetraborate
  - Trichloroacetic acid (TCA)
  - Methanol
  - Deionized (DI) water
- Buffer
  - TCA buffer: 0.04 mol L^{-1} sodium tetraborate, adjusted to pH 8.5 with 70% w/v TCA (for fortified flour extraction)

[1] Alaburda J, de Almeida A, Shundo L, Ruvieri V, Sabino M. Determination of folic acid in fortified wheat flours. Composition and Analysis. 2008;21:336-342. doi:doi:10.1016/j.jfca.2007.12.002

    - Acetate-phosphate buffer - pH 4.5, 0.1 mol/L sodium acetate, adjusted with acetic acid containing 5% dibasic potassium phosphate (for elution of FA from SPE Column)
- Samples
- Food processor, lab blender, or sample mill
- Immersible sample homogenizer
- Rotational shaker or ultrasonic bath
- Centrifuge with capacity for 50 mL tubes
- 50 mL centrifuge tubes
- 0.45 um nylon membrane filter
- Solid-phase extraction (SPE) Anion-exchange columns (SAX, 500 mg/3mL)
- 5-mL volumetric flask
- HPLC system equipped with multi-solvent pumping system, degasser, autosampler, column oven, computer software, and ultraviolet detector
- Reversed-phase column protected with a guard column

Folic acid standard solution:

1. Create a stock standard solution of FA with 0.1 mol L^{-1} NaOH and FA standard (DSM Nutritional Products)
2. Prepare a standard sample series for a calibration curve, using appropriate volumes of the stock solution diluted with acetate-phosphate buffer (pH 8.5)

Sample preparation:

- Corn masa flour –
    - Add 5g of maize flour sample to 50 mL of tetraborate-TCA buffer pH 8.5 in an Erlenmeyer shaker flask
    - Shake for 30 min. in rotational shaker or place in ultrasonic bath for same time period
- Corn masa (dough) –
    - Homogenize 5 g of sample with 50 mL of tetraborate-TCA buffer pH 8.5 in blender/homogenizer until homogenously dispersed
    - Transfer to flask and shake for 30 min. in rotational shaker or place in ultrasonic bath for same time period
- Tortillas or other solid foods –

- Pulverize sample in food processor or mill
- Homogenize 5 grams of sample with 50 mL of tetraborate-TCA buffer pH 8.5 in blender/homogenizer for 3 min. at room temperature and maximum velocity
- Transfer to flask and shake for 30 min. in rotational shaker or place in ultrasonic bath for same time period

Extraction:

1. Transfer sample to a 50 mL centrifuge tube
2. Centrifuge 5 min at 3500 rpm
3. Filter supernatant through preconditioned SPE column:
    a. Condition SPE column with 5 mL of methanol and 5 mL of DI water
    b. Apply 2.5 mL of the supernatant and elute at a rate of about 0.5 drop/sec.
    c. After extract is fully eluted, rinse SPE with 5 mL of DI water and drain
    d. FA is then eluted from the SPE column with 5 mL acetate-phosphate buffer into a 5 volumetric flask which was then brought to volume.
    e. Filter the eluate through a 0.45 um nylon membrane filter

Chromatography conditions:

Quantify FA using high-performance liquid chromatography (HPLC) system equipped with a multi-solvent pumping system, a degasser, column oven, and an ultraviolet detector (set to 280 nm for FA detection).

A Supelcosil LC 18 reversed-phase column (Supelco) 250mm X 4.6mm, 5 mm) protected with a Phenomenex C18 (4mm X 3.0mm, 5 mm) guard column is recommended by Alaburda, et al.. Maintain the column temperature at 35°C. Use a gradient elution with ACN and AA 1% (pH 2.8), flow rate 0.7mL/min. For the gradient start at 8% (v/v) ACN, and after 10 min raise the ACN proportion linearly to 26%. After maintaining the 26% concentration gradient for 5 min, raise the ACN concentration linearly to 50% within 6 min, and maintain the 50% concentration for 9 min, after which the ACN proportion is reduced back to 8% over 5 min so that the column can be restabilized for 10 min before the next injection.

Quantification:

1. Create calibration curve by injecting aliquots from the standard sample series prepared as indicated above and plotting HPLC peak areas against their known concentrations.
2. Quantify fortified sample FA by correlating peak areas with concentrations on the standard curve and applying appropriate dilution factors based on the extraction and separation steps.
3. Linearity was determined in the range of 6-600 ng/mL, FA retention time was 14.5±0.2 min.