AOCS/SQT Amino Acid Round Robin Study

J. V. Simpson and Y. Zhang
National Corn-to-Ethanol Research Center
Edwardsville, IL

Richard Cantrill
Technical Director
AOCS, Urbana, IL

Amy Johnson
Soybean Quality Traits Program (SQT)
AOCS, Urbana, IL

Lars Reimann
Eurofins Scientific, Inc.
Memphis, TN
Phase I Study  
08/2009 - 06/2010

Goal of study can be described in two parts:

1. Determine the major differences between the various amino acid (AA) determination methods in terms of accuracy, reproducibility, and repeatability.
   
   • This will be determined independent of amino acid hydrolysis.

2. Make recommendations and improvements to existing methods through the cumulative data collected from all study participants.
Phase I Study
08/2009 - 06/2010

AA testing, Two steps

AOAC method for acid hydrolysis (AOAC 994.12)
Instrumental quantitation of hydrolysate (HPLC, UPLC, LC/MS/MS, etc.)

Experimental Design
18 samples for each lab
15 amino acids to report (ug / ml) (alanine, arginine, aspartic acid, glycine, glutamic acid, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine and valine)

Accuracy (AA-standard (Sigma), NIST food reference material, animal feed and spiked animal feed samples)
Precision (within-batch and between-batch)

Participants
14 labs
Instrument and Derivatization Utilized

- Lab A - HPLC Post-Column
- Lab B - HPLC Post-Column
- Lab C - UPLC Pre-Column
- Lab D - HPLC Post-Column
- Lab E - HPLC Post-Column
- Lab F - HPLC Pre-Column
- Lab G - HPLC Pre-Column
- Lab H - HPLC Pre-Column
- Lab I - HPLC Post-Column
- Lab J - HPLC Post-Column
- Lab K - UPLC Pre-Column
- Lab L - LC/MS/MS Pre-Column
- Lab M - HPLC Post-Column
- Lab N - HPLC Post-Column

HPLC Post

HPLC Pre

UPLC Pre

LC/MS/MS Pre
Sample Preparation

Samples prepared using AOAC method for acid hydrolysis (AOAC 994.12) from:

- Soybean meal
- DDGS provided by the AAFCO Check Sample program
- Swine starter provided by the AAFCO Check Sample program
- Poultry feed provided by the AAFCO Check Sample program
- Infant/adult nutrition formula (NIST SRM 1849)
AOCS AA Experimental Design

Accuracy
- Sigma AA std.
  - Independent of sample matrix and systematic error
- NIST Infant Formula
  - With sample matrix and independent of systematic error
- Spiked Hydrolysates
  - With sample matrix and systematic error

Precision
- 3 replicates
  - Within batch, repeatability
- 6 measurements over 6 weeks
  - Between batch
Accuracy (Recovery, %)
(Based on Sigma amino acid standard)
Accuracy (Recovery, %)
(Soybean meal hydrolysate spike)

Post-HPLC | Pre-HPLC | Pre-UPLC | LC-MS/MS

% Recovery

Lab A B D E I J M N F G H C K L

Ala Arg Asp Gly Glu ILue Lue Lys Phe Pro Ser Tyr Val
Comparison of Soybean hydrolysate
(mean of triplicates)

Post-HPLC

Pre-HPLC

Pre-UPLC

LC-MS/MS

Amino acid content (µg/mL)

Lab  A   B   D   E   I   J   M   N   F   G   H   C   K   L

Ala  Arg  Asp  Gly  Glu  ILue  Lue  Lys  Phe  Pro  Ser  Thr  Tyr  Val
Precision (RSD, %)
(Soybean meal hydrolysate, over 6-week period)

% RSD

Post-HPLC  Pre-HPLC  Pre-UPLC  LC-MS/MS

Lab    A    B    D    E    I    M    N    F    G    H    C    L

Ala  Arg  Asp  Gly  Glu  His  ILue  Lue  Lys  Phe  Pro  Ser  Thr  Tyr  Val
Comments

Significance of error in the data:
• Error between labs may be greater than the error between methods
• Masks the error between methods

All labs should include a reference material in every batch of samples, such as NIST SRM

Use of an internal standard should be included in the sample pretreatment phase, after hydrolysis.
Phase II Study
08/2010 - 03/2011

Goal of study can be described in two parts:

1. Determine the major differences between the various hydrolysis procedures used by each lab participant that affect accuracy and repeatability.

2. Make recommendations and improvements to existing hydrolysis procedures through the cumulative data collected from all study participants.
Experimental Design

- Each lab participant will receive 5 animal feed samples (including NIST SRM) and hydrolyze each sample once a week for three weeks.

- All the hydrolysate samples will be collected and tested for 15 amino acids by one participating lab in the U.S.

People interested in participating should contact Amy Johnson, AOCS
amyj@aocs.org
217-693-4820