INTRODUCTION

Amino acid analysis is used for the quantification of one group of essential nutrients in foods and feeds. To ensure the quality and safety of the food and feed products, it is essential to determine the content of essential amino acids in the materials. The amino acid analysis is also used for the quantification of all the amino acids, both sample and standard, in the chromatographic separation, which must be considered in consideration. In sample handling, the samples must be hydrolyzed so that the proportions of amino acids can be measured. Multiple hydrolysis approaches must be used because all amino acids are not equally resistant to acid or base hydrolysis. The application of microwave hydrolysis for all three protocols resulted in improved control of hydrolysis conditions with better accuracy, reproducibility, speed and robustness. In this work we show the implementation of all three hydrolysis protocols for raw feeds, such as, soy bean meal, as well as complete characterization, Table 2.

RESULTS

Derivatization Chemistry

A. Performic Acid Oxidation (Pre-oxidation):

B. Acid Hydrolysis:

C. microwave hydrolysis can be used with all three hydrolysis protocols for raw feeds.

METHODS

Sample Handling

A. Performing Acid Oxidation (Pre-oxidation):

B. Acid hydrolysis under basic conditions

C. microwave hydrolysis used with all three hydrolysis protocols for raw feeds.

Optimization of Hydrolysis Protocols

Hydrolysis conditions must be optimized for improved processing. The samples were subjected to varying hydrolysis time with constant temperature. The samples were also subjected to hydrolysis at different temperatures while holding the time. Hydrolysis conditions were varied between 210°C for 30 minutes. The optimization experiments are summarized in Figure 7a and 7b.

Complete Nutritional Analysis

Table 1. Normalized weights of amino acids with respect to phenylalanine during hydrolysis optimization experiments.

Table 2. Normalized weights of amino acids with respect to phenylalanine during hydrolysis optimization experiments.

Figure 1. Microwave Hydrolysis Procedure

Figure 2. Amino acid analysis system

Figure 3. AcceTag Ultra Eluent B

Figure 4. Reaction of AQC reagent with amino acids. The 6-aminohexanoyl-H-3-formimidoyl-3-carboxylic acid (AQC) reagent reacts with both primary and secondary amines.

Figure 5. Food and feeds Standard with line added as an internal standard. Differences on coelution.

Figure 6a. Effect of varying time of hydrolysis while keeping temperature constant at 195°C. 6b. Effect of varying temperature while keeping time constant at 30min.

Figure 7. Total amino acids in mg/g of solid feed: 7a. when the time is varied keeping temperature constant at 195°C. 7b. when the temperature is varied keeping time constant at 30min.

CONCLUSIONS

Three protocols are required to describe completely the nutritional content of a sample. Microwave hydrolysis can be used with all three hydrolysis protocols. Acid hydrolysis optimization experiments take 24 hours to 55 minutes. The combination of microwave hydrolysis and AQC amino acid analysis improves throughput for nutritional analysis of feeds to approximately 30 samples in a 24 hour period.