

Acetone Precipitation

## Measurement of Peptide Fractions and Soluble Proteins Found in MOTIV

#### **OVERVIEW**

MOTIV® is a unique protein hydrolysate derived from the fermentation of corn proteins with *Lactobacillus spp.* It is through this fermentation that the proteins are broken down into smaller subunits allowing for the production of a multichotomous variety of free amino acids (FAA) and peptides of different sizes. To better understand the range of peptides that are found in MOTIV an analysis was performed.

#### **OBJECTIVE**

The objective of this work was to measure the peptide fractions found in MOTIV and soluble proteins through untargeted proteomics analysis.

#### **MATERIALS AND METHODS**

- MOTIV samples were extracted at ambient temperature in water and processed in a series of steps illustrated in Figure 1.
- Extracts were then filter-fractionated into three molecular weight cut-offs (MWCOs) utilizing the Pall MWCO filters in the 1k, 3k, and 10k ranges.
- All fractions were measured with the Thermo Scientific<sup>™</sup>
   Colorimetric Peptide Assay (and the concentrations (µg/
   ml) were used to determine the net peptide percent
   corresponding to every sample's MWCO (<1KDa, 1-3KDa,
   & 3-10Ka).</li>
- In addition to peptide quantification, total soluble proteins were measured using internal methods and FAA were measured externally at NP Analytical Laboratories (St Louis, MO).

# Motiv Supernatant 200µL of FT 300µL of cold acetone 1.5ml LBMT 2ml in every tube 200µL of FT 300µL of cold acetone 1.5ml LBMT 10 min, 14K g 10 min,

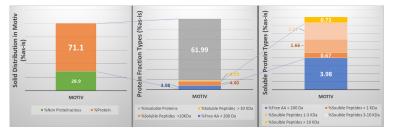
FIGURE 1. Process flow to measure peptide fraction sizes.

### **RESULTS**

Overall, the free amino acid (FAA), peptide fractions, and soluble protein results for each production run were averaged, charted, and concluded that we have the following distributions of proteinaceous solubles:

Proteinaceous Fractions	M.W.	as % of total solids (as-is)
%Free AA	< 200 DA	3.98
%Soluble Peptides	< 1 KDa	0.67
	1-3 KDa	1.66
	3-10 KDa	2.07
	> 10 KDa	0.73
%Insolubule Protieins		61.99

Measurements of soluble and insoluble protein/peptide breakdown in the Motiv<sup>™</sup> product and their corresponding molecular weight cutoffs (Averages of 15 production runs are reported):



#### **SUMMARY**

When looking at the protein/peptide breakdown in the fermented protein hydrolysate, MOTIV, it is important to note the following:

- Peptides with <1KDa MW are a mix of di- and up to penta-peptides.
- Peptides with 1-3 KDa MW are a mix of up to 20-AA peptide chain.
- More than 500 peptides have been identified on HRAM LCMS and they are present at different concentrations in MOTIV.

