2002 MAFMA Final Report

Project Title: **BIOACTIVE PROTEINS IN INDUSTRIAL SOY STREAMS**

PI **Elvira de Mejia**
Co-PI **Benito de Lumen**

Academic Institution **University of Illinois**

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1. Objective Summary

The objective of this proposal is to perform a preliminary screening of bioactive proteins with nutraceutical properties in industrial soy streams.

2. Objective Accomplishments

We accomplished the following objectives:

- Detection and quantification of lunasin from different industrial soy stream fractions.
- Isolation and purification of lunasin from the most promising industrial soy streams.
- Development of an enzyme-linked immunosorbent assay (ELISA) method to identify lunasin.
- Quantification of lunasin in selected diverse soybean accessions.

Lunasin is a unique and novel cancer preventive peptide originally isolated from soy. Information on lunasin concentration of soybean cultivars and commercial soy proteins would be useful in developing lunasin-enriched cultivars and soy products. We report the development of an enzyme-linked immunosorbent assay (ELISA) method to identify lunasin and quantify the variations in concentration in 144 selected, diverse soybean accessions from the U.S. Department of Agriculture Soybean Germplasm Collection, several commercially available soy protein fractions and isoflavone-enriched products. With synthetic lunasin and monoclonal antibody, ELISA shows a linear concentration range of 24-72 ng/mL, good reproducibility, a detection limit of 8 ng/mL, and a recovery of 90% on spiked soy samples. Lunasin concentrations in the tested materials range from 0.10 to 1.33 g/100 g flour. Differences that exceeded 100% have been observed among accessions of similar maturity that were grown in the same environment, indicating that genetic differences in soybeans exist for lunasin. The mean of 23 major ancestral lines of U.S. cultivars is similar to the mean of 16 modern cultivars selected to represent the current diversity of the crop, but the highest values were found within the ancestral and exotic accessions. Soy protein concentrate, isolate, and hydrolyzate contain 2.81 ± 0.30, 3.75 ± 0.43, and 4.43 ± 0.59 g lunasin/100 g flour, respectively, while soy flour and soy flakes contain 1.24 ± 0.22 g lunasin/100 g flour. Isoflavone-enriched products contain very little or no lunasin. The relative mass (M(r)) of lunasin in the samples is 5.45 ± 0.25 kDa. The wide range of lunasin concentrations within the Glycine max species indicates that the levels of this important bioactive peptide can be genetically manipulated. Furthermore, soy isolates and hydrolyzed soy proteins contain the highest concentrations of lunasin.

A soy saponin concentrate and a textured soy concentrate had insignificant amounts of lunasin. A lunasin-enriched flour presented the highest concentration of 27.3 mg/g solid material. The

evidence gathered so far on the health benefits of isoflavones has led to the appearance of soy isoflavone-enriched products on the market. These products contain very little or no lunasin except for two products (Soy Care for Menopause and Low Isoflavone), which have values in the low range of lunasin contents found in the soy protein products. This is likely due to the low solubility of lunasin in ethanol, which was used to extract isoflavones.

3. Unexpected findings, if any

- Interestingly, the Bowman-Birk inhibitor null accession PI 321393 *Glycine latifolia* contains no lunasin.
- The variations in lunasin concentration observed in a large number of cultivars indicate that the levels of lunasin in soybeans can be genetically manipulated.
- It is also clear that large-scale processing of soy, to produce different soy protein fractions, influences lunasin concentration.

4. Practical impacts of research efforts.

a. Short Term Impacts

Soy molasses were found to contain 1.72 mg/g solids, an amount eight times less than the average for soy flour and soy flakes. We have developed an inexpensive process for isolation and purification of lunasin from soy molasses in waste down-streams. The anion exchange resin Dowex 1x 4 is charged positively, which allows lunasin (which is negatively charged, due in part to its high number of aspartic acid residues) to bind during the loading of the sample. The wash with buffer flushes most of the contaminants allowing for a more specific elution with NaCl. This elution step was performed in several trials with 0.7, 0.9, 1.2 and 1.5 M NaCl. A washing step with 1.5 to 2.0 M NaCl after elution was needed in order to obtain a recovery of up to 85% of lunasin.

Ion exchange can be used in the purification of lunasin from waste streams, separating the color contaminants and eluting lunasin with a recovery of 60% that can be increased up to 85% by increasing the elution bed volumes.

b. Long Term Impacts

Among the U.S. cultivars, Cisne had the lowest and Kunitz had the highest concentration of lunasin. Both lines were developed at the University of Illinois. Kunitz is a near-isogenic line developed through five cycles of backcrossing with Williams-82 as the recurrent parent and PI 157440 as the donor parent of the *ti* allele, which eliminates the Kunitz trypsin inhibitor. By pedigree, Williams provides half of the genes to one of the parents of Cisne. PI 240664 has a much higher lunasin concentration than any of the other lines and is the only line in these samples that was grown in a subtropical environment. The large differences in lunasin concentration that we observed among these lines suggest that real genetic differences do exist. These results also suggest that over 50 years of selection for high yield and desirable agronomic traits has had little effect on lunasin concentration and that modifying lunasin concentration among high-yielding cultivars should be possible.
The results could expand the uses of soybean for health and medical purposes.

Further pursuit by PI of research in area of this project:

"Characterization of Bioactive Peptides in *Amaranthus hypochondriacus* Seed Storage Proteins"

5. Publications resulting from this research.

Gonzalez de Mejia, E., Vasconez, M., de Lumen, B., Nelson, R. Lunasin content in different genotypes of soybean seeds and commercial products. *J. Agric. Food Chem. 52,* 5882-5887, 2004.

de Mejia, E., Vasconez, M., Wang, W., Nelson, R., O. De Lumen, B. Physiologically Active Peptides in Soybean and Soy Products. *Nutrition and Physiological Functionality. VII World Soybean Research Conference*. IV International Soybean Processing and Utilization Conference, Brazil, March 1, 2004.

de Mejia, E., Soy Bioactive Peptides. A New Frontier In Soybean Utilization. Workshop on Functional Properties of Soybean derivatives: New Perspectives. *International Life Sciences Institute*. Ministry of Health Brazil. Brasilia, Brazil, March 5, 2004.

M.Vasconez, R. Nelson and E. Gonzalez de Mejia. Effect of genotype and environmental growing conditions of soybean on the concentration of lectin and lunasin. Institute of Food Technologists 85-8: 70. July 2005. New Orleans.