

2002 MAFMA Final Report

Project Title **Improving the flavor of soy protein as a food ingredient**

PI **Gary Reineccius**

Co-PI **Lloyd Metzger**

Academic
Institution **University of Minnesota**

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1. Objective Summary

1) To develop an accurate means of measuring the binding potential of a wide range of aroma compounds to soy proteins; and 2) Determine the factors that influence the binding potential of these aroma compounds to soy proteins.

2. Objective Accomplishments

The primary focus of this project was to apply Isothermal Titration Calorimetry (ITC) to study binding that occurs between a range of aroma compounds and soy proteins. ITC is used routinely in biophysical research to study the solution energetics of biopolymer-molecular interactions and thus, it appeared useful in our application. Prior research on milk protein binding constants by O'Neill and Kinsella (1987) gave us a seemingly good starting point to develop an ITC method. We chose BiPro (Davisco Foods International, LeSeuer, MN), which is composed mostly of beta-globulin (β -LG), to develop a method to ultimately use for soy protein products (e.g. whey protein concentrates and isolates). In this method, a BiPro solution (20mM) is used to "titrate" a solution of aroma compounds for study. In this work we used 2-heptanone (877.19 mM), 2-octanone (781.25 mM), or 2-nonanone (704.22 mM). Numerous different configurations of reactants and instrumental parameters were tested, but to no avail, we were unable to obtain usable data. For example, Figure 1 shows ideal ITC data (obtained from MicroCal website) where the heat released during each injection of protein drops drastically after a certain amount of injections. This drop indicates that the protein binding sites are 'full' and thus no more heat released. For an undetermined reason, our peaks never dropped. Our data indicated that some other persistent interaction was occurring beyond the estimated useable ranges that were based on published K_A data. Usable peak data normally translate into an isotherm (Figure 1) that ultimately gives binding constants (K_A), reaction stoichiometry (n), enthalpy (DH) and entropy (DS).

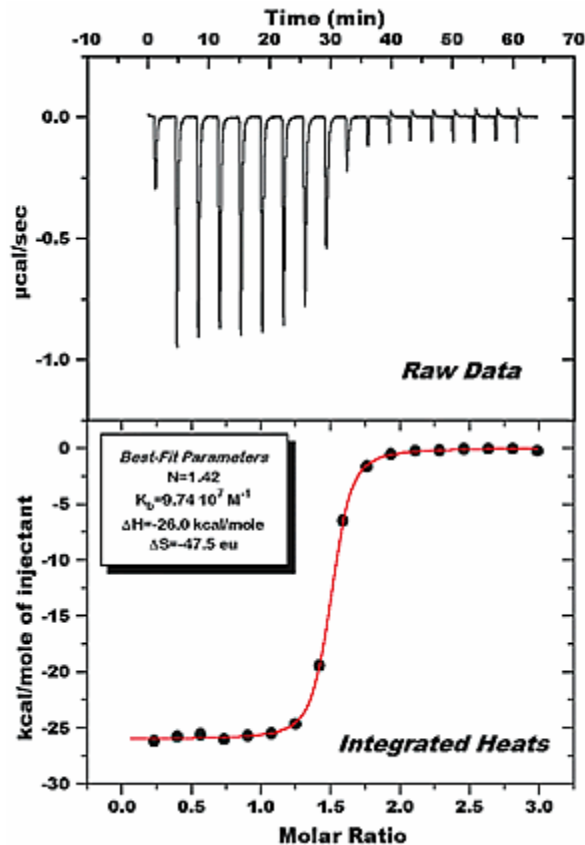


Figure 1. Ideal ITC data.

Since some interaction (besides binding) was occurring, we assumed that our protein was not pure. After consultation with Dr. David A. Bernlohr (Professor of Biochemistry and Molecular Biology and a frequent user of ITC), we suspected that our protein sample was contaminated with hydrophobic residues. We spent considerable time trying to remove all hydrophobic material from our protein samples focusing on solid phase extraction (SPE). Our approach involved passing a BiPro protein solution through a column of Lipidex 5000 (Catalog # 6008305, Perkin Elmer, Boston, MA) to strip the protein of hydrophobic molecules. The collected solution was freeze-dried and prepared for ITC analysis. Even after this purification, we were unable to obtain usable data.

We then decided to obtain “pure” soy protein fractions from another research institution. ‘Pure’ samples of glycinin and β -conglycinin (the major soy globulins) were procured from the Iowa State University pilot plant. After much testing, these pilot plant samples were still not ‘pure’ enough to run on the ITC. Soy proteins exist in numerous genetic variants and configurations (hetero-trimers) and we decided that it would not be possible for us to isolate individual proteins of adequate purity to be tested by ITC: the characterization and purification techniques required were beyond the scope of this project.

After investing significant effort in attempting to apply ITC to measuring flavor:soy protein interactions, it has failed to yield the data needed to determine binding energies between these components. While this is not a desirable outcome, it is a reality that necessitated our

considering alternative methods to measure soy protein/aroma interactions. We chose to use an equilibrium headspace method as the alternative method. Equilibrium headspace, while not providing the depth of information that would be available by ITC, is commonly used and is well accepted for studying food ingredient/aroma compound interactions.

We chose a simple model aroma system for study consisting of compounds that may be used to impart a desirable flavour to a soy product (i.e. 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal) and those associated with an undesirable flavour (i.e. hexanal, dimethyl trisulfide and 2-pentylfuran). (The model compounds we used in ITC study were chosen because there were data on soy:flavor compound binding in the literature for these compounds) We then added these compounds at typical food concentrations to a commercial soy protein isolate dispersion, allowed time for equilibration, and then analyzed the headspace for the model volatiles. A reduction in headspace concentration of volatiles (Vs. pure water) reflected the interaction occurring between the soy proteins and the volatiles, i.e. decreased headspace concentrations indicated flavour binding.

We felt that the primary factors we might change to influence flavor:protein binding were pH (pH 3.0, 4.5 and 7.0) and salt concentration (50 and 200 mM). These factors may be changed during processing and then changed back for use. These changes are likely to be reversible so that the protein would maintain its characteristic functional properties. While it is well documented that the soy protein fraction, and thermal processing history both have a strong influence on flavor:protein binding, these factors are not necessarily free to be changed for our purposes (or are reversible) and thus, were not studied.

Our results indicated that soy protein isolate reduced the headspace concentration of all of the volatiles studied (indicating binding to proteins) and the magnitude of binding was compound dependent (which was anticipated). There was no influence of salt concentration or pH on the binding of soy protein with hexanal or dimethyl trisulfide. However, there was a very pronounced decrease in binding of soy proteins with 2-pentyl furan (considered primarily responsible for the beany note of soy products) in the presence of salt and binding decreased with increased salt level. Thus, it appears that soy products could be deodorized more effectively (at least the beany note removed) if salt is added prior to any deodorization treatment.

When we consider the binding of desirable flavor components, neither salt nor pH had a major effect. The trend was that the desirable flavor components studied were bound to the greatest extent at pH 4.5 – close to the isoelectric point of the soy. This suggests that soy would have the greatest ability to change food flavor at this pH.

3. Unexpected findings, if any

We did some work using sensory panels to determine if chocolate flavor was more effective at masking beany flavor or beany flavor more effective at masking chocolate flavor. It is of interest that chocolate is often used to attempt to cover up off flavors so we wanted to know how effective chocolate is in this respect when used in soy products. Our sensory results demonstrated that in using chocolate flavor (model systems) and in real products (ice cream), chocolate was less effective at masking the beany note than the beany note in masking chocolate flavor. This does not support the idea that chocolate is an ideal masking flavor in soy products. This result was unexpected.

I guess we could note that we really expected the ITC to yield useful data – it did not.

4. Practical impacts of research efforts.

a. Short Term Impacts

The short term impact is that there appears to be little value in attempting to use ITC in this type of flavor application. We need to use other methodologies for this purpose.

b. Long Term Impacts

I believe the most useful results of this work are:

1) the indication that some aspects of beany flavored soy products can likely be most effectively removed by deodorization treatments in the presence of salt. This is likely to improve the sensory quality of soy flavored foods; and

2) chocolate may not be the best flavor for trying to cover up beany flavored soy. We suggest that other flavors that are more compatible with the indigenous beany note be used.

6. Publications resulting from this research.

Two publications are in progress. One deals with the use of ITC for the project and the other on the alternative method used to gather the needed information. The proper credit will be given to USDA for funding the work.