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Rebuttal on A Semi-Pilot-Scale Procedure for Isolating and Purifying Soybean (*Glycine max*) Lectin

Sir: The following is a rebuttal to Dr. Liener's comment regarding our paper published recently in the *Journal of Agricltural and Food Chemistry* (*1*). Dr. Liener comments that the semi-pilot-scale procedure (based on affinity chromatography and ultrafiltration) we designed for the isolation and purification of soybean lectin (SBL) offered little improvement, if any, over the techniques (fractionation with ammonium sulfate and ethanol) used in his 1953 research (*2, 3*). We disagree with his assertion and propose that our procedure offers a clear improvement in the purity and quality of the SBL obtained and has the potential to be readily scaled up to produce larger amounts of the lectin in less time. These points are addressed below.

Affinity chromatography is a purification method based on biospecific interactions such that only the biologically active forms of a protein are obtained. In contrast, the ammonium sulfate and ethanol precipitation techniques used by Liener (*2*, *3*) are not specific for native forms and may denature some proteins (*4*). Thus, we believe that our affinity-purified SBL would contain more native forms of the lectin if compared to the lectin preparation obtained by Liener in 1953. Native electrophoretic analysis followed by Western blotting with SBL-specific antibody clearly showed that our affinity-purified SBL contained more of the native lectin compared to the denatured forms (*1*).

In terms of purity, sodium dodecyl sulfate−polyacrylamide gel electrophoretic analysis followed by Western blotting with SBL-specific antibody confirmed that our affinity-purified SBL was completely homogeneous. In contrast, the lectin fraction of the preparation obtained by Liener in 1953 was only 78% homogeneous and contained some trypsin inhibitor activity and possibly lipoxidase (*2*, *3*). Methods based on solubility differences under various conditions almost never give preparations with a purity greater than ∼90% when examined by the more sensitive analytical methods.

It took us 3 weeks to purify 5.4 g of SBL from 3480 g of soyflakes, whereas Liener obtained 3 g of the lectin preparation in 10 days. Scaling up the affinity chromatography step, which is the most time-consuming stage in our procedure, could have shortened the time we used to purify our SBL. The affinity chromatography stage is readily scaled up by increasing the resin binding capacity through the addition of more *N*-acetyl-D-galactosamine resin (purchased from Sigma Chemical Co., St. Louis, MO). When money is the constraint, as was in our case, one could personally prepare the *N*-acetyl-D-galactosamine resin (*5*−*7*). The effect of increasing the resin binding capacity cannot be underestimated. For example, tripling the amount of resin in the column (from 225 to 675 mL) in our procedure (*1*) should reduce the time taken to complete the purification process by 6 days and subsequently result in the production of 5.4 g of SBL in 15 days. Thus, it is certainly possible to better the time scale (purifying 3 g of SBL in 10 days) reported by Liener without altering any other stage in our procedure or significantly increasing cost.

It is our opinion that our large-scale procedure is superior to the one described by Dr. Liener in 1953 because the SBL we obtained was of higher purity and quality. In addition, our procedure is easily scalable and eliminates the risk of using flammable chemicals in a large-scale process as required by Liener's procedure.

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