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Comment on Extraction and Purification of Isoflavones from Soybeans and Characterization of Their Estrogenic Activities

The recently published paper by Zhang et al. (*1*) describes a method for the extraction and quantitation of isoflavones from soybean flour, in addition to comparing estrogenic activity using the E-SCREEN (*2*). Although the paper was well written in many respects including literature review and method development, certain omissions leave the reader with more questions than should be reasonably expected.

A critical omission was found under Method Validation of HPLC Analysis (p 6943). The reader is referred to Figure 2 of the Supporting Information, but at the time of this writing (October 1, 2007, original placed on Web July 21, 2007) there was no Supporting Information for this paper online. Because the whole premise of this paper is method validation for quantitation, it is critical to have access to calibration curves. Table 1 does report regression equations and R^2 values for these curves, AS, LODs, and LOQs for each aglycone isoflavone, but the concentration of standards included is unknown. After inquiry to the journal, Supporting Information is now available (October 8, 2007).

In Figure 5, chromatograms are presented of isoflavone product before optimization (A) and after (B). The extraction process "before optimization" is not defined. Does this refer to product extracted with 100% ethanol, stirring for 2 h at 37 °C, followed by centrifugation? If so, then it could be assumed that the increase in absorbance units obtained "after optimization" is a reflection predominantly of the hydrolytic processing of glycones to aglycones, rather than an enhanced extraction efficiency. In the discussion of optimization an improvement in yield of isoflavones is cited, without "change in the product composition". Whereas the authors go to great length in the introduction to discuss differences in concentrations, bioavailability, and relative estrogenic activity of isoflavones in the glycosylated or aglycosylated state, they fail to quantitate just what percentage change in yield is due to hydrolysis (with conversion to an aglycone) versus extraction efficiency. This could have been addressed by subjecting one set of samples to hydrolysis and one not. In the discussion the authors state "In this study, >85% of genistin and daidzin was degraded by hydrolysis, whereas the concentrations of genistein and daidzein were increased about 40-fold (details not shown)." The lack of data makes it impossible to assess what percentage of isoflavones in the unextracted sample was glycosylated.

In the evaluation of "our extracts" the authors seem to draw implications that are not supported by the data presented. For instance, "according to the HPLC chromatogram (Figure 5b), the composition of our product was relatively simple." Compositional complexity of a sample would not be evaluated from such a limited HPLC gradient (30−50% methanol, monitoring absorbance at 255nm) to claim "only minor impurities detected in this product." Taken together, the implication is that there are no major "impurities" or other compounds in this product. Such implications are worrisome, as these products are intended for human consumption.

In the comparison of "our product" to four other commercial products tested, all data presented in Figures 5 and 6 are apparently from a single extraction of their product. The authors base their claims of superior estrogenic activity over three of the four other supplements tested on the "MTT assay (results not shown)". One large paragraph goes on to generally describe the results—with no data given—yet *P* values are included. In conclusion, although the paper provided a nice description of a method, it falls short in providing much needed information about the composition of isoflavones found in commercial products and the relative merits of the new extraction procedure.

LITERATURE CITED

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