ISOFLAVONE FINGERPRINTING OF SOY-BASED NUTRITIONAL SUPPLEMENTS VIA ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY

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As intrinsic to *Leguminosae* with particular regard to soybean, isoflavones comprise a class of weak-estrogenic, polyphenolic *phyto*-chemicals that are mainly credited for their health promoting effects especially associated with certain hormone dependent cancers, cardiovascular diseases and osteoporosis. Concerning their biomedical attributes, a vast multitude of commercial soy-based nutritional supplements has been widely emphasized as a complementary alternative to hormone replacement therapy, promising safe remedies for alleviating menopausal symptoms. However, prevalent nutritional information tend to label solely the total isoflavone contents, whereas the specific isoflavone conjugates as well as the applied calculation basis, whether referring to bioactive aglycone equivalents or the conjugated glycoside forms, often remain unclear.

Hence, commercially available soy-based nutritional supplements were characterized via a newly established ultra performance liquid chromatography (UPLC™) method, on both their native conjugated isoflavone spectra subsequent direct extraction, as well as on quantitative amounts derived as total aglycones after enzymatic hydrolysis utilizing *Helix pomatia* juice.

Capitalizing on *sub-2 μm* particles, the established RP-UPLC™ technique facilitated an efficient chromatographic separation of all 12 soy intrinsic isoflavone forms within 10 minutes, thus demonstrating vast improvements compared to conventional HPLC techniques in particular regarding enhanced resolution, sensitivity and speed. Derived native isoflavone profiles after solvent extraction implied a certain variability, comprising conjugated forms, especially glycosides, as the predominant isoflavonic constituents throughout the majority of supplements, whereas only two samples

indicated the more bioavailable free aglycones as prevailing compounds. Concerning possible instabilities as well as inter-conversions especially of esterified isoflavone glycosides during sample preparation, thereby implicating variable profiles, an enzymatic hydrolysis towards stable aglycones ensures a more accurate option for quantification. Incubation with *β-glucuronidase*, inherent in *Helix pomatia* juice, in consideration of adequate organic solvent concentration to maintain isoflavone solubility without inhibiting enzyme activity, yielded in a complete de-conjugation, indicating exclusively aglycone residues with no traces of glycoside- or esterified conjugates. Moreover, the robust quantification as total aglycones subsequent to enzymatic hydrolysis unexceptionally yielded negative deviations referring to the labeled specifications, thus implying that stated amounts were typically calculated on basis of the high molecular isoflavone conjugates. Hence, incorporation of those higher molecular weights consequently yielded in considerable higher virtual isoflavone contents.

This in fact will complicate any consumer decision that is basically relying on the labeled content itself, hence pointing out the urgent need for more transparent specifications and an uniform basis for calculating the labeled isoflavone contents, thus permitting better comparability of these nutritional supplements.