# Bioaccessability of isoflavone aglycones from soy food is enhanced by bile salts during *in vitro* digestion

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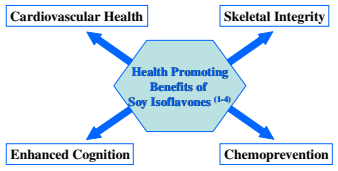
## ABSTRACT

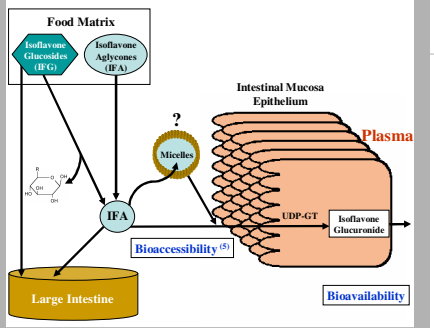
Isoflavone (IFN) aglycones, but not glucosides, are preferentially transferred across the apical membrane of enterocytes despite their relative insolubility in an aqueous milieu. Here we investigated the impact of bile extract (BE) on bioaccessibility of IFN from a soy food. Soy bread was subjected to simulated oral, gastric, and small intestinal digestion. The aqueous (bioaccessible) fraction was isolated from digesta by centrifugation and analyzed by HPLC.

IFN glucosides and aglycones were stable during simulated digestion with recoveries from soy bread exceeding 98%. Partitioning of aglycones, but not glucosides or malonyl glucosides, into the aqueous fraction was affected by BE concentration during simulated small intestinal digestion. Transfer of aglycones into the aqueous fraction at standard BE concentration (2.4 mg/mL) was < 65%. Omission of BE resulted in absence of genistein and < 45% of total daidzein, glycitein, and acetylgenistin present in the aqueous fraction. With BE at 4.8 mg/mL, 102, 54, 120, and 79 % of daidzein, genistein, glycitein, and acetylgenistin, respectively, partitioned into the aqueous fraction. Results suggest that micellarization is required for optimal IFN aglycone bioaccessibility in the small intestine, and that bioavailability of IFN from foods will exceed that of supplements due to enhanced bile secretion in response to fat and protein ingestion.


## INTRODUCTION




### Digestion and Absorption of Soy Isoflavonoids In The Upper GI Tract




### Table 1. Chemical Speciation of Isoflavonoids

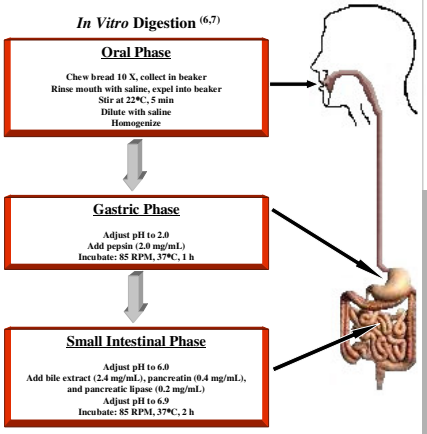
| Daidzein Family | Genistein Family | Glycitein Family |
|---|---|---|
| Daidzein | Genistein | Glycitein |
| Daidzin | Genistin | Glycitin |
| Acetyldaidzin | Acetylgenistin | Acetylglycitin |
| Malonyldaidzin | Malonylgenistin | Malonylglycitin |

## SPECIFIC AIM 1

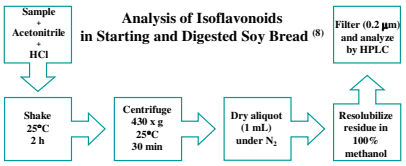
**Are isoflavonoids from soy bread stable during simulated oral, gastric, and small intestinal phases of digestion?**

## MATERIALS & METHODS

### *In Vitro* Digestion (6,7)




### Analysis of Isoflavonoids in Starting and Digested Soy Bread (8)

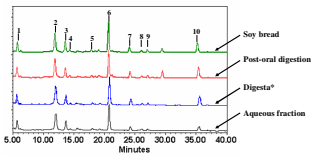



| HPLC SYSTEM | |
|---|---|
| Column | 3.9 x 150 mm Nova-Pak C18 |
| Separation | Waters 2695 Separations Module |
| Detector | Waters 2996 Photodiode Array (PDA) |
| Software | Empower |
| **HPLC CONDITIONS** | |
| Flow Rate | 0.6 mL/min |
| Injection Volume | 10 µL |
| Run Time | 50 min |
| PDA | 210 – 400 nm (λ max = 260 nm) |
| Column Temp. | 25°C |

| SOLVENT GRADIENT | | |
|---|---|---|
| Time (min) | % A (1% Acetic Acid) | % B (Acetonitrile) |
| 0 | 85 | 15 |
| 5 | 85 | 15 |
| 36 | 71 | 29 |
| 44 | 65 | 35 |
| 45 | 85 | 15 |

## RESULTS

**Figure 1.** Reverse-phase HPLC analysis of isoflavonoids in starting and digested soy bread.

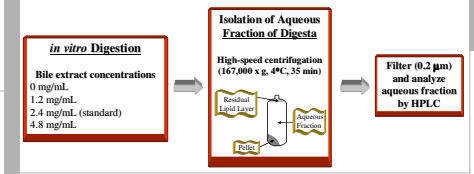



*Digesta: Soy bread sample that was subjected to simulated oral, gastric, and small intestinal digestion.

## SPECIFIC AIM 2

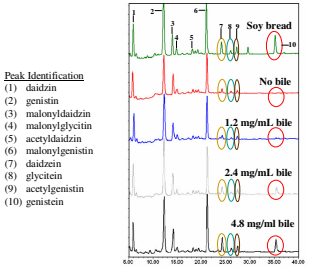
**Does low solubility of isoflavonoid aglycones necessitate micellarization for optimal bioaccessibility in the small intestine?**

## MATERIALS & METHODS



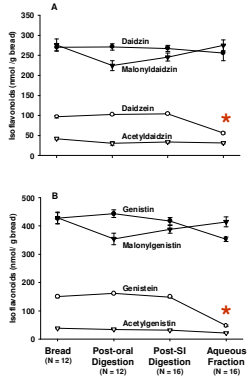

## RESULTS

**Figure 3.** Reverse-phase HPLC analysis of the aqueous fraction of digesta following *in vitro* digestion with varying bile concentration.



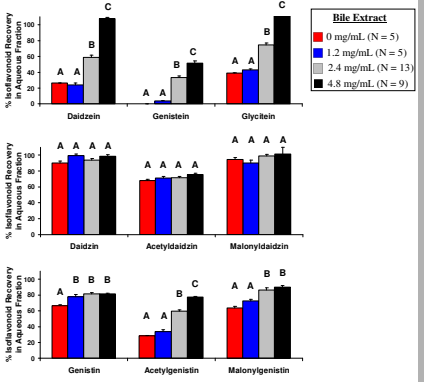

Note that the area under the curve for daidzein (gold circle), genistein (red circle), glycitein (teal circle) and acetylgenistin (brown circle) in the aqueous fraction increases as the concentration of bile extract present during the small intestinal phase of digestion is increased.

**Figure 2.** Isoflavonoids are stable during simulated oral, gastric, and small intestinal digestion. The amount of daidzein, genistein, and acetylgenistin in the aqueous (bioaccessible) fraction of digesta was significantly ($P < 0.01$) less than that in the digesta.




* Significantly different ($P < 0.01$) from the starting soy bread meal. Data are means ± SE.

**Figure 4.** Bile enhances partitioning of daidzein, glycitein, acetylgenistin, and genistein into the aqueous (bioaccessible) fraction of the digesta. Partitioning of conjugates of daidzein and glycitein were not affected by the concentration of bile extract during simulated small intestinal digestion.




Data are mean percentages ± % SEM. Means within a group that do not share a common letter above bars differ significantly ($P < 0.01$) from one another as determined by one-way analysis of variance (ANOVA) followed by Bonferroni post-hoc comparison of statistical significance.

## SUMMARY

In general, isoflavonoids from soy bread were stable during simulated oral, gastric, and small intestinal phases of digestion (figures 1 & 2).

A considerable percentage of isoflavonoid aglycones and acetylgenistin in the digesta were absent from the aqueous bioaccessible fraction (figures 1 & 2).

Micellarization during the small intestinal phase of simulated digestion appears to enhance the bioaccessibility of isoflavonoid aglycones and acetylgenistin (figures 3 & 4).

Partitioning of malonylglycitin and the glucoside conjugates of daidzein was independent of the bile extract content during the small intestinal phase of simulated digestion (figures 3 & 4).

### CONCLUSION

Bioavailability of isoflavonoids from foods containing fat and protein may exceed that of isoflavonoid supplements consumed without food due to enhanced bile secretion.


## ACKNOWLEDGEMENTS

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