The Effect of In Vitro Soy Bread Digestion on Isoflavone Profile

Y.C. Zhang[1], K.R. Walsh[2], M.L. Failla[2], S. J. Schwartz[1], and Y. Vodovotz[1]

ABSTRACT

Isoflavones present in soy have been associated with a decrease in risks of certain cancers. Baked products, such as bread, made partly with soy ingredients, offer an attractive isoflavone delivery system due to their popularity in the Western diet.

An in vitro digestion method serves as a model that emulates in vivo digestive processes thereby providing an indication of components available for intestinal absorption. Our objective was to determine the effects of in vitro digestion on the quantity and the relative stability of three isoflavone daidzein, genistein, and glycitein present in soy bread.

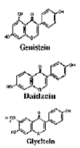
Soy bread was homogenized and diluted in saline then subjected to a two-stage in vitro digestion method. This method involved acidification and incubation with pepsin (gastric phase) followed by neutralization and incubation with pancreatin, pancreatic lipase,and bile extract (small intestinal phase). The isolated aqueous micellarized fraction, containing the components which are readily available for absorption, was isolated by centrifugation. Reverse-phase HPLC was used to determine the isoflavone concentration and composition of these samples after acidified acetonitrile extraction.

Undigested samples contains 557.9 μg of total isoflavones per gram. Similar profiles of isoflavones were found in the digested aqueous phase. However, the aqueous phase contained lower quantities of isoflavones as compared to the amount of isoflavone present in the undigested samples. Aqueous phase micelles were place on Caco-2 cells to measure the extent of uptakes into the intestinal epithelial cells.

Reduction of isoflavone concentrations in the aqueous phase and Caco-2 cells after soy bread digestion may lead to alterations in intestinal uptake and bioavailability.

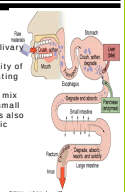
INTRODUCTION

Isoflavones



- Diphenolic compounds naturally occurring in plants, highest in the soybean plant.
- A Class of phytoestrogens, bear a structure similar to mammalian estrogen.
- Major isoflavones: Genistein, Daidzein, Glycitein and their glycosidic conjugates.
- Epidemiological studies indicate that dietary isoflavones provide health benefits for men and women.
- The bioavailability of dietary isoflavones have been linked to their chemical forms.

Human Digestive System



- Digestion is initiated in the oral cavity when food is masticated and mixed with salivary secretions.
- The acidic gastric milieu facilitates continuity of digestion by denaturing proteins and activating pepsin.
- Gastric contents are neutralized when they mix with alkaline pancreatic secretions in the small intestinal (SI) lumen. Pancreatic secretions also contain enzymes that catalyze the hydrolytic degradation of protein, lipid, and starch.
- Bile secreted into the SI lumen emulsifies lipophiles.
- Nutrient absorption involves transport of hydrophillic moieties across the unstirred water layer and concomitant enterocyte uptake.
- Components remaining in the SI lumen ultimately pass to the colon.
- Resident microbes can alter substrates present in the colon.
- The dynamic environment of the digestive tract plays a vital role in the bioavailability of isoflavones ingested with soy bread.

EXPERIMENTAL DESIGN

I. Isoflavone Stabiliy During Digestion

What is the digestive stability of isoflavones during oral, gastric, and small intestinal digestion?

METHODS

In vitro Digestion of Bread

Oral Phase

Chew bread, expel into collection beaker
Rinse mouth with saline, expel into collection beaker
Stir at 22 ºC, 5min
Homogenize

Gastric Phase

Adjust pH to 2.0 with 1M HCL
Pepsin (40mg/mL)
Incubate at 37ºC, 1hr.

Small Intestinal Phase

Adjust pH to 6.0 with 1M $NaHCO_3$
Bile extract (40mg/mL)
Pancreatin (10mg/mL)
Pacncreatic lipase (5mg/mL)
Adjust pH to 6.9 with 1N NaOH
Incubate at 37ºC, 2hr

Digesta* — *Sample subjected to simulated gastric and small intestinal phases.

Ultracentrifugation

167,000 x g, 4ºC, 35min

Lipid fraction | Aqueous fraction | Pellet

Isoflavone Extraction

HPLC Analysis of Isoflavones in extracts

Column:	3.9 x 150 mm Nova-Pak C18
Separation:	Waters 2695 separation model
Detector:	996 photodiode array
Mobile Phase:	A - 1.0% acetic acid in water B - 100% acetonitrile
Flow Rate:	0.6mL/min
Run Time:	50 minutes

RESULTS & DISCUSSION

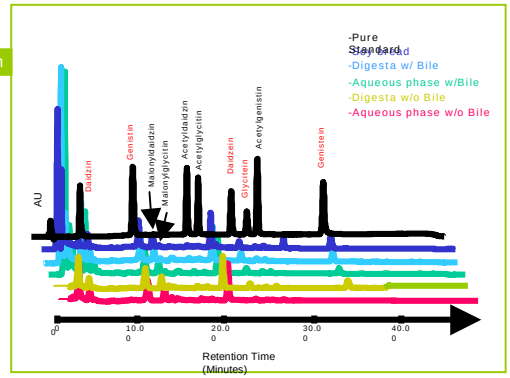


Figure 1. Reversed-phase HPLC -UV (260 nm) chromatography of of extracts of soy bread and its digestive samples.

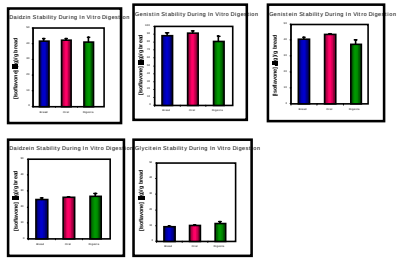


Table 1. Recovery of isoflavones from digesta

Isoflavones	Soy Bread (μg/g)	Digesta (μg/g)	% Recovery
Daidzin	41.80	40.90	97.8%
Genistin	87.95	81.34	92.5%
Malonyldaidzin	84.00	65.14	77.6%
Malonylglycitin	18.50	14.16	76.6%
Acetyldaidzin	13.24	3.10	23.4%
Acetylglycitein	17.15	N/D	N/D
Malonylgenistin	169.95	172.37	101.4%
Daidzein	24.71	26.66	107.9%
Glycitein	9.21	11.27	122.4%
Acetylgenistin	7.15	2.50	35.0%
Genistein	40.59	37.31	91.9%
Total	514.24	454.76	88.4%

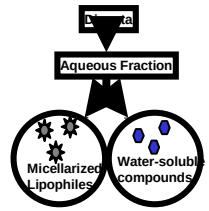
N/D: Not detectable

- There were no significant differences in daidzin, daidzein, genistin, genistein, or glycitein concentrations between in vitro digesiton phases (p-value < 0.05, one-way ANOVA).

II. Bioaccessibility of Isoflavones

•What is the bioaccessibility* of isoflavones after *in vitro* digestion of soy bread?

Bioaccessibility: The portion of compounds in digesta present in the aqueous phase that is available for uptake across the brush-border membrane of the enterocyte.



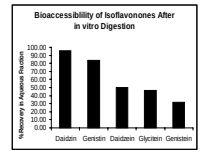


Figure 1 – Partitioning of aqueous fraction

- For daidein and genistein, significant difference in content was particularly found between aqueous phase and the other three types of digestion samples. The levels in aqueous was significantly decreased from digesta to aqueous by 50% for daidzein and 70% for genistein in average. Reasons that caused this 'loss' need to be further investigated. Preliminary data from experiments involving varied amounts of bile suggest that micellarization of genistein and daidzein may be necessary for maximum bioaccessibility.

Part III - Isoflavone Micellarization

The digesta samples with and without bile contained the same levels of isoflavones, while the aqueous phase of the digesta without bile was different from the other two. In the aqueous phase without bile, the isoflavone aglycone levels decreased significantly, esp. for genistein, which was not detectable any more.

The obvious appearance of genistein in digesta and the disappearance of it in aqueous phase may indicate the bile's role in the bioavailability of isoflavones, such as incooperating isoflavone aglycones into the micells to make their further uptake by enterocytes possible.

CONCLUSIONS

➔ Daidzin, genistin, and their respective aglycones remain stable during *in vitro* digestion with some 'loss' in the aqueous phase.

➔ Levels of glycitin were too low to detect in the samples. Glycitein remains stable during *in vitro* digestion with some loss in the aqueous phase.

➔ It is probable that the lower concentration of isoflavones in the aqueous phase is due to protein and/or carbohydrates interactions rather than isoflavone degradation.

➔ The existence of bile in small intestine is necessary to incooperate isoflavone aglycones into the aqueous phase by micellarization and to make their uptake by enterocytes possible.

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[1]Department of Food Science and Technology, [2]Department of Human Nutrition, The Ohio State University, Columbus, OH