Torsten Bohn

Isoflavone bioavailability from foods and supplements

Dietary factors impacting utilization

TORSTEN BOHN

Centre de Recherche Public Gabriel Lippmann
Department of Environmental and Agro-Biotechnologies
41, rue du Brill, Belvaux, L-4422, Luxembourg

ABSTRACT: Isoflavones comprise a group of phytoestrogenic polyphenols with antioxidant properties occurring especially in plants of the Leguminosae family, and are consumed by humans mostly in form of soy (Glycine max (L.) Merr.) and products. Soy rich isoflavone products have been promoted recently as foods or supplements with functional or nutraceutical characteristics, especially for the prevention of osteoporosis, for improved cardio-vascular function, and against prostate and breast cancer. However, there is controversial discussion on the bioavailability of isoflavones, a prerequisite for their action in the human body, depending potentially on the type of isoflavonoid, its chemical binding state (aglycon vs. glucosides), and the food matrix, such as the presence of dietary fibre. In addition, dosing and factors altering gut microflora, such as prebiotics, have been suggested to affect utilization of isoflavones.

KEYWORDS: isoflavonoids, health, supplements, food matrix, bioavailability.

INTRODUCTION

Isoflavonoids or isoflavones are plant polyphenols chemically similar to the parent compound isoflavone, occurring predominantly in several *Fabaceae* varieties, including psoralea, kudzu, fava- and soy bean, and in a variety of nuts, such as peanuts (Table 1). In terms of human food sources, soybeans constitute the major dietary source, with concentrations of isoflavones typically ranging between 1-30 mg/100g (1), however, higher concentrations up to 155mg/100g can be found (Table 1). In Western countries, the intake of isoflavones from dietary sources is estimated at <1 mg/d, while in Asian countries, much higher amounts are consumed within the diet, approx. 20-100mg/d (2).

The most abundant isoflavones are the aglycons genistein, daidzein, glycitein, the genistein precursor biochanin, the daidzein precursor formononetin, and their respective conjugates, the β-glucosides, the 6''-O-acetyl-β-glucosides, and the 6''-O-malonyl-β-glucosides (Figure 1). While in the native soybean, isoflavones are present mainly in form of the malonylgucosides followed by β-glucosides (1), heating or fermentation can lead to higher amounts of the free, more hydrophobic aglycons. Different isoflavone composition patterns may be important, as these can impact the amount of isoflavones absorbed, kinetics of absorption and excretion, and also metabolisation, such as the formation of microbacterial metabolites, e.g. equol, which have been suggested, based on high antioxidant properties and estrogenic activities, to possess health benefits, possibly even superior compared to the native isoflavones (3).

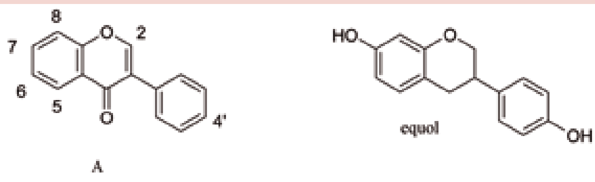
However, especially soy and soy products rich in isoflavones have been advertised as health promoting agents, for delaying or preventing of a number of chronic diseases, including cardiovascular complications, hormone related cancers, especially breast and prostate, and osteoporosis (3, 4). A health claim, but only with respect to soy protein (i.e. for products containing at least 6.25 g per serving), was approved by the US Food and Drug Administration for its cholesterol lowering effect. Indeed, it has been suggested that the combination of both soy protein and isoflavones results in higher health beneficial effects (2). While information on the content of soy isoflavonoids is available, the data situation on isoflavone bioavailability is scant. However, as will be shown in this review, it has been suggested that factors including composition of soy isoflavones and food matrix could impact uptake and use of isoflavones by the human body, and should therefore be of interest for several stakeholders, including consumers and the food industry.

Table 1. Occurrence and concentrations of isoflavones in plants used for human nutrition.

plant, food source^	isoflavone concentration (mg/100g)*	predominant isoflavones§
Kudzu (*Pueraria lobata*)**	200	daidzein (puearin+), genistein
Alfalfa sprouts (*Medicago sativa*)**	130	formononetin
Clover sprouts (*Trifolium pratense*)**	100	formononetin, genistein, biochanin A, daidzein
Breadroot (*Psoralea esculenta*)	10 - 100	daidzein, genistein
Soy bean (*Glycine max*)	60 - 155	genistein, daidzein, glycitein
Lentils (*Lens culinaris*)	10	formononetin
Lupin beans (*Lupinus spp.*)	10	genistein
Peas (*Pisum sativum*)	2.4	genistein, daidzein

Data based on USDA database (http://www.nal.usda.gov/fnic/foodcomp/Data/isoflav.html), ^raw, mature unprocessed condition, * per 100 g edible content, **animal feed,drugs, supplements, +daidzein-8-C-glucoside, § aglycon form specified only.

Figure 1. Major isoflavones, their respective glucosides (A), and equol, a daidzein intestinal bacterial metabolite.



compound	Position of rest					
	2	5	6	7	8	4'
isoflavone	-H	-H	-H	-H	-H	-H
daidzein	-H	-H	-H	-OH	-H	-OH
genistein	-H	-OH	-H	-OH	-H	-OH
glycitein	-H	-H	$-OCH_3$	-OH	-H	-OH
biochanin A	-H	-OH	-H	-OH	-H	$-OCH_3$
formononetin	-H	-H	-H	-OH	-H	$-OCH_3$
Daidzin**	-H	-H	-H	-O-glucose	-H	-OH
6-O''-acetyldaidzin	-H	-H	-H	-O-glucose-$COCH_3$	-H	-OH
6-O''-malonyldaidzin	-H	-H	-H	-O-glucose-$COCH_2COOH$	-H	-OH

**analogous substitutions fof genistein, glycitein.

ISOFLAVONE RICH FOODS AND SUPPLEMENTS

Among the most sold isoflavone containing products range probably soy protein isolate, soy flour, soy milk, tofu, tempeh (a fermented product rich in aglycons), soy nuts, and various isoflavone supplements (5, 6). A variety of functional foods rich in isoflavones have also been developed, ranging from several breakfast cereals, energy and snack bars, frozen desserts to soy-germ fortified tomato juice (7). Soy foods and beverages are a fast growing market, with sales approx. in the area of 4 billion USD in the US in 2008, and estimated annual growth rates of 6-15 percent (e.g. http://www.soyfoods.org/products/sales-and-trends). Among these, isoflavone containing dietary supplements, with over 20 million USD in 2004 in the US, have become an important market. However, many of these supplements have been suffering from poor characterization in terms of amount isoflavone present, and form, all of which could impact bioavailability. Variations between isoflavone content can be large, but are typically in the area of approx. 10-60 mg isoflavones per capsule (5, 6). Isoflavone supplements are usually produced based on soy such as soy germ containing high amounts of glucosides and are relatively rich in glycitin, or are made from other plants, such as clover or kudzu (6), containing higher amounts of formononetin and biochanin A. In order to improve water solubility of isoflavones (8, 9) or to delay their release (10), microencapsulations have been developed. However, the composition of supplements and the ratio of glycosides to aglycons has been shown to vary considerably (6).

A number of dietary factors have been suggested to impact isoflavonoid bioavailability, however, more studies in this area are needed

Dietary factors potentially impacting isoflavone bioavailability include dietary fiber, dosing, and type of isoflavone ingested

BIOAVAILABILITY AND METABOLISATION

In order to estimate isoflavone bioavailability, it is important to understand the metabolisation pathways occurring in the human body following ingestion. Isoflavone absorption from food typically ranges between 10-50 percent (2). As isoflavone glycosides cannot be absorbed intact in significant amounts (11), these have to be cleaved to release the aglycons. This cleavage can either occur by enzymes present in the food, by bacterial enzymes in the small or large intestine (2), or by human enzymes in the gut wall, such as membrane bound lactase phlorizin hydrolase (LPH) (12). It is not sure which of these mechanisms are predominating, but it has been shown that antibiotic treated individuals (13) and ileostomists without intact large intestine (14) are able to absorb isoflavones to similar extents as healthy subjects, indicating that cleavage by enzymes present in the food and gut wall allow for high uptake of isoflavones. However, ileostomists and antibiotic treated persons did show reduced formation of isoflavone metabolites (13, 14). For example, ortho-desmethylangolensin (ODMA) and equol can be formed out of daidzein by microbial metabolisation in the large intestine, and 6-OH-ODMA and p-ethylphenol from genistein.
While glycosides are probably easily transported to their site of deglycolysation due to their hydrophilicity, for the more hydrophobic aglycons, micellarisation is needed for optimal availability (15). However, aglycons can finally be taken up by the ephitelial cells, both in the small and the large intestine, probably by non-saturable passive diffusion. They are then glucuronidated and or sulfated to increase water solubility, this process happens to a large extent already in the intestine and later also in the liver and kidney (2). They are then, in the glucuronidated and/or sulfated form, transported via the blood stream into various tissues, while free aglycon concentrations remain relatively low (2). Isoflavones have been detected in the prostate and breast, often in concentrations exceeding that of plasma (16). Only small amounts are finally excreted in the feces (<5 percent), reflecting either non-absorbed or re-excreted isoflavones via e.g. bile and pancreas, which is partly due to re-absorption, i.e. enterhohepatic recirculation (2). The majority of isoflavones is excreted in the urine, up to ca. 50 percent of isoflavones ingested, mostly as glucuronides or glucuron-sulfates, with highest plasma clearance rate and excretion for daidzein and glycitein and lowest for genistein (2).

ISOFLAVONE DOSING AND BIOAVAILABILITY

For low to medium concentrations, until ca. 35 mg intake, it has earlier been proposed that isoflavone absorption is linearly related to the given dose (17), i.e. following a non-saturable absorption process such as passive diffusion, suggesting also no significant competition between isoflavones for absorption. Medium to higher concentrations however, above ca. 30-40 mg, have meanwhile shown in several studies (e.g. 18, 19) to go along with decreased fractional absorption compared to lower doses. It has been hypothesized that the rate limiting step of absorption is the transfer of the isoflavone from the intestinal bulk to the cell wall (2). Thus, it appears that at low concentrations this is indeed the rate-limiting step of absorption, but that at higher concentrations, other mechanisms also become limiting factors, such as the cleavage rate of the glucosides into the aglycon.

TYPE OF ISOFLAVONES IMPACTING BIOAVAILABILITY

An impact exists of the type of the isoflavone and its binding state, i.e. free aglycon vs. glucoside on the bioavailability. As aglycons do not require further cleavage, these are the most readily available forms for absorption, with uptake starting in the proximal small intestine. Consequently, this form is also less likely to undergo significant bacterial metabolisation prior to absorption. This is in contrast to the glucosides, which may be, at least partly, cleaved at the more distal parts of the small or even large intestine,

allowing for additional bacterial metabolisation. Even though it is estimated that only about 30-40 percent of the population is able to produce equol, (20), it can be assumed that other bacterial products are formed, such as ODMA, and that these are then available for absorption in the large intestine. Thus, isoflavones present in the more native, glucoside forms, are a) absorbed slightly less rapidly compared to their aglycons (21); b) metabolized into more metabolites (22), and therefore c) appear later in the plasma with a longer half-life, however, the overall bioavailability (especially when taking metabolite forms into account) does not seem to differ between both forms in the majority of carefully conducted studies (19, 22, 23), see also Table 2. It can be speculated that the disadvantage of requiring cleavage prior to absorption is somewhat counterbalanced by a more rapid diffusion of the glucosides to the epithelial cells, due to their higher water solubility. In addition, reduced matrix binding and protective effects of the glucoside forms have also been discussed (24).
In contrast to the somewhat controversial aglycon/glucoside discussion, genistein has been consistently reported of having the highest bioavailability based on dose-corrected appearance of plasma values, followed by daidzein, with a lack of data for glycitein (25). The reasons are not fully understood, but it is possible that the more polar daidzein is absorbed and excreted more rapidly, as suggested by its higher urinary recovery rates. It is also possible that daizein is more prone to bacterial metabolisation than genistein and therefore less available for direct absorption or absorption following enteric or enterohepatic recirculation, or that genistein glucosides are faster hydrolyzed by LPH (12).

Compared to isoflavonoid aglycons, glucosides can be assumed to be absorbed less rapidly, be metabolized into more metabolites, but possess possibly similar bioavailability

There is currently limited information that apart from liquid vs. solid foods and the form of isoflavones, i.e. aglycons vs. glucosides, matrix does have a large impact on bioavailability. For example, bioavailability of the daidzein and genistein precursors formononetin and biochanin A from red clover vs. soy were comparable (28), as was isoflavone bioavailability from biscuits vs. cereal bars (29) and from soymilk powder vs. soy germ (30). However, studies in this area are lacking.
Some dietary components have been suggested to potentially alter isoflavone bioavailability. It has been discussed whether prebiotics could alter isoflavone bioavailability, as these nonfermentable carbohydrates may stimulate fermentability and metabolisation of isoflavones. This has been suggested for fructooligosaccharides in rats (31), and for inulin in humans (32), and a trend for higher isoflavone and metabolite bioavailability was observed following ingestion of resistant starch and probiotics (33).

FOOD FACTORS IMPACTING ISOFLAVONE BIOAVAILABILITY

As the food matrix can be related to gastrointestinal passage time, and prolonged passage time and/or delayed release of isoflavones has been suggested to increase bacterial metabolites, matrix is expected to impact bioavailability of isoflavones. For example, in a recent study, isoflavone absorption from a liquid juice was faster compared to cookies and chocolate bars (26), similar as for a beverage compared to capsules (27) which was explained by faster stomach emptying and gut transit time. These results are in agreement with the majority of studies investigating soy drinks (Table 2). Whether this rapid absorption goes along with higher bioavailability is questionable, especially when comparing matrices with similar levels of aglycons to glycosides over sufficiently long plasma appearance. A complex matrix that slows down gastro-intestinal (GI) passage time has even been suggested to improve isoflavone bioavailability (21), but this remains to be elucidated in further studies.

Dietary factor	Major findings regarding bioavailability
Dietary fiber	Negative impact in few studies.
High dosing	Negative effects in most of a dozen studies, especially above 30-40 mg.
Liquid matrix	Reduced bioavailability or no effect in several studies.
Supplements vs. complex food matrix	Unclear, some of the few studies suggesting higher, some lower absorption. Results confusing due to confounding factors.
Higher bioavailability of glucosides vs. aglycons	Many of the over a dozen studies showing no effect, some suggesting higher, some lower absorption. Results confusing due to confounding factors, e.g. different matrix, varying amounts of isoflavones.
Higher bioavailability of genistein vs. daidzein	Higher bioavailability of genistein in several studies, due to faster clearance and excretion of daidzein.
Fermentable fiber	Trends for increased absorption and increased metabolite formation in a few studies.

Table 2. Dietary factors impacting isoflavone bioavailability.

BIOAVAILABILITY FROM SUPPLEMENTS

Whether supplements possess better isoflavone bioavailability compared to food items is controversial. While higher bioavailability was found from supplements as opposed to soy cheese (5), higher bioavailability was suggested from various soyfoods vs. tablets (34). In the first study, the amount glycosides vs. aglycons remains uncertain, in the second this was similar between the soyfood and the tablets. It is not always possible to compare studies, as some trials base bioavailability on a single bolus dose, while others investigate bioavailability following a longer dietary intervention. It deems possible that prolonged ingestion increases absorption of isoflavones originating from glycosides such as through increased activity of beta-glucosidase in the intestine.
However, as soy products do not necessarily rank high in the consumers' perception in the Western Culture, isoflavone supplements are regularly used, and likewise ways seeking to improve the absorption of isoflavones.

Microencapsulation with modified cellulose for improved solubility and delayed release of isoflavones and altered metabolisation was tried, resulting in higher daidzein compared to genistein absorption, with reasons being unclear (10). Another way to increase bioavailability seems by complexation with beta-cyclodextrins (35) or by incorporation into pluronic micelles (36). Another possibility rests in the chemical modification of the isoflavones, e.g. in form of daidzein 7-O triglucosides (37).

CONCLUSIONS

A number of dietary factors have been shown to alter isoflavone bioavailability, including the type of isoflavone and the presence of dietary and fermentable fibre, while others such as aglycon vs. glucoside form, and liquid vs. solid foods are still under discussion. Future studies in this area are warranted with respect to the several potential health beneficial effects of isoflavones, and to improve the quality and characterization of isoflavones in foods and supplements.

REFERENCES AND NOTES

1. L. Coward, N.C. Barnes et al., *J Agric Food Chem.*, **41**, pp. 1961-1967 (1993).
2. T. Larkin, W.E. Price et al., *Crit Rev Food Sci Nutr.*, **48**, pp. 538-552 (2008).
3. J.P. Yuan, J.H. Wang et al., *Mol Nutr Food Res*, **51**, pp. 765-781 (2007).
4. A.O. Omoni, R.E. Aluko, *Nutr Rev.*, **63**, pp. 272-283 (2005).
5. S. Vergne, C. Bennetau-Pelissero et al., *Br J Nutr.*, **99**, pp. 333-344 (2008).
6. K.D. Setchell, N.M. Brown et al., *J Nutr*, **131**, 1362S-75S (2001).
7. S. Tiziani, Y. Vodovotz, *J Agric Food Chem.*, **53**, pp. 7267-7273 (2005).
8. J.S. Seok, J.S. Kim et al., *Arch Pharm Res.*, **26**, pp. 426-431 (2003).
9. H. Wu, C. Lu et al., *Drug Dev Ind Pharm.*, **35**, pp. 138-144 (2009).
10. K.D. Setchell, A. Brzezinski et al., *J Agric Food Chem.*, **53**, pp. 1938-1944 (2005).
11. K.D. Setchell, N.M. Brownet al., *Am J Clin Nutr.*, **76**, pp. 447-453 (2002).
12. A.J. Day, F.J. Canada et al., *FEBS Lett*, **468**, pp. 166-170 (2000).
13. A.A. Franke, B.M. Halm et al., *Nutr Cancer*, **60**, pp. 627-635 (2008).
14. K.R. Walsh, S.J. Haak et al., *Am J Clin Nutr.*, **85**, pp. 1050-1056 (2007).
15. K.R. Walsh, Y.C. Zhang et al., *J Agric Food Chem.*, **51**, pp. 4603-4609 (2003).
16. A. Cassidy, *J AOAC Int*, **89**, pp. 1182-1188 (2006).
17. S.C. Karr, J.W. Lampe et al., *Am J Clin Nutr.*, **66**, pp. 46-51 (1997).
18. K.D. Setchell, N.M. Brown et al., *J Nutr.*, **133**, pp. 1027-1035 (2003).
19. D. Tsangalis, G. Wilcox et al., *Br J Nutr.*, **93**, pp. 867-877 (2005).
20. J.L. Slavin, S.C. Karr et al., *Am J Clin Nutr.*, **68**, 1492S-1495S (1998).
21. I.L. Nielsen, G. Williamson, *Nutr Cancer*, **57**, pp. 1-10 (2007).
22. L. Zubik, M. Meydani, *Am J Clin Nutr.*, **77**, pp. 1459-1465 (2003).
23. M. Richelle, S. Pridmore-Merten et al., *J Nutr.*, **132**, pp. 2587-2592 (2002).
24. C.E. Rufer, A. Bub et al., *Am J Clin Nutr.*, **87**, pp. 1314-1323 (2008).
25. T. Izumi, M.K. Piskula et al., *J Nutr.*, **130**, pp. 1695-1699 (2000).
26. S. de Pascual-Teresa, J. Hallund et al., *J Nutr Biochem.*, **17**, pp. 257-264 (2006).
27. E. Anupongsanugool, S. Teekachunhatean et al., *BMC Clin Pharmacol.*, **5**, p. 2 (2005).
28. N. Tsunoda, S. Pomeroy et al., *J Nutr.*, **132**, pp. 2199-2201 (2002).
29. B. Chanteranne, F. Branca et al., *Clin Interv Aging*, **3**, pp. 711-718 (2008).
30. Y. Zhang, G.J. Wang et al., *J Nutr.*, **129**, pp. 957-962 (1999).
31. M. Uehara, A. Ohta et al., *J Nutr.*, **131**, pp. 787-795 (2001).
32. C. Piazza, M.G. Privitera et al., *Am J Clin Nutr.*, **86**, pp. 775-780 (2007).
33. T.A. Larkin, W.E. Price et al., *Nutrition*, **23**, pp. 709-718 (2007).
34. C.D. Gardner, L.M. Chatterjee et al., *J Nutr Biochem.*, **20**, pp. 227-234 (2009).
35. S.H. Lee, Y.H. Kim et al., *Biosci Biotechnol Biochem.*, **71**, pp. 2927-2933 (2007).
36. S.H. Kwon, S.Y. Kim et al. *Arch Pharm Res.*, **30**, pp. 1138-1143 (2007).
37. D. Li, J. H. Park et al., *J Agric Food Chem.*, **52**, pp. 2561-2567 (2004).