British Journal of Nutrition (2003), **89**, Suppl. 1, S39–S43
DOI: 10.1079/BJN2002795

Food sources of phyto-oestrogens and their precursors in Europe

R. J. Fletcher*

Kellogg Company, The Kellogg Building, Talbot Road, Manchester M16 0PU, UK

Phyto-oestrogens are dietary components found in some plants, which act *in vivo* like weak oestrogens. They may reduce the risk of some degenerative diseases moderated by oestrogen, including breast cancer and osteoporosis. The most widely studied are the isoflavones genistein and daidzein from soyabeans, but lignans may be more prevalent in the European diet. Soya foods have traditionally been consumed in the Orient for millennia, and are now widely available to European consumers. Levels of isoflavone in soyabeans from published literature vary between 560 and 3810 mg/kg, depending on variety and growing conditions. Soya protein concentrates and isolates derived from soyabeans contain 466–615 mg isoflavones/kg. Traditional soya milk, bean curds, bean sprouts, etc. contain 13 to 2030 mg isoflavone/kg, depending on the starting raw material and final water content. Fermented foods have a different isoflavone conjugate profile, which may be important in absorption and metabolism. Soya analogues of European foods include dairy and meat products, which contain 38 to 3000 mg total isoflavones/kg, depending the source of soya and dilution with other ingredients. A wide range of foods contain low levels of soya-derived isoflavones, but such foods do not make a significant contribution to mean intakes in Europe. Flaxseed is by far the richest source of lignan precursors. However, foods such as cereal brans, legumes and some vegetables are a more important source in the diets of Europeans because they are more widely consumed. For similar reasons, compared with soya isoflavones, lignans may be a more important source of phyto-oestrogens in the diets of Europeans.

Phyto-oestrogens: Isoflavones: Soya: Food

Introduction

Phyto-oestrogens are naturally occurring plant components, which are chemically similar to mammalian oestrogens (Setchell & Adlercreutz, 1988). Their weak oestrogen-like properties have attracted mounting attention over recent years, because of their potentially beneficial health effects (Cassidy *et al.* 2000; Mazur & Adlercreutz, 2000; van der Schouw *et al.* 2000).

Wider awareness of their possible health benefits has also led to the growing interest of consumers, and consequently to a greater range of both traditional and new phyto-oestrogen-rich products on the market. This has occurred in Europe, but to a greater extent in the USA.

The isoflavones genistein and daidzein, the major phyto-oestrogens in soya, have received by far the greatest attention; however, other phyto-oestrogens such as lignans may also be of importance to public health. This paper reviews the phyto-oestrogen-rich foods available to the European consumer. Whilst actual intakes are beyond the scope of this paper, recent estimates of isoflavone intake in Ireland, Italy, The Netherlands and the UK (van Erp-Baart *et al.* 2003) and Finland (Valsta *et al.* 2003) are available elsewhere in this supplement.

Soya isoflavones

Isoflavones are natural components of legumes, including black beans, green split peas and clover sprouts. However, their concentration in soyabeans is two orders of magnitude higher than in other legumes. Because of this, soya-based foods are the main source of isoflavones in the Western diet (Mazur, 1998; Liggins *et al.* 2000*a*,*b*). Soya isoflavones occur in two main biologically active forms, genistein and daidzein. Both of these are present in soya-derived foods, either as aglycones or as glycosides together with acetic and malonic acid moieties.

Soyabeans and their derivatives

The concentration of total isoflavones in the raw soyabean varies widely due to differences in genetics, cultivar, climate, location and agricultural practices (Farmakalidis & Murphy, 1985; Wang & Murphy, 1994*b*; Liggins *et al.*

Abbreviations: VENUS, Vegetal Estrogens in Nutrition and the Skeleton.

* **Corresponding author:** Dr R. J. Fletcher, fax +44 (0)161 869 2103, email reg.fletcher@kellogg.com

2000*a,b*), and values ranging between 580 and 3810 mg/kg have been reported (Reinli & Block, 1996). While heat treatment appears to change the relative proportions of the different glycosides, mainly by conversion of malonyl and acetyl glycosides to the more stable non-acylated β-glycosides, the total isoflavone content is relatively unchanged by cooking (Barnes *et al.* 1994). Therefore roasted beans may contain high levels of total isoflavones because of their reduced water content, but with a wide range due to the factors mentioned above (Table 1) rather than to heat degradation.

Soya oil is produced by extraction from whole beans, typically using hexane extraction or by pressing. Isoflavones are not soluble in hexane or lipids and therefore soya oil contains no isoflavones, unlike the residual oil-depleted soya flakes, which are a rich source. These are the raw material for the protein concentrates and isolates used in the manufacture of many ‘second-generation’ soya foods.

The processing techniques used to produce commercial soya protein concentrates and isolates are extremely varied. This diversity of processing techniques has a major effect on the level of isoflavone in the individual, final food ingredient. Soya protein concentrates are made from dehulled and defatted soyabeans, by either water or alcohol extraction of proteins. They contain at least 65 % protein on a moisture-free basis.

Soya protein isolates are produced in a similar way, but have a higher concentration of protein because more of the non-protein and fibrous components are removed by water or mild alkali extraction. They contain at least 90 % protein on a moisture-free basis.

Textured soya protein is made from soya flour, soya protein concentrates or soya isolates, by extrusion and subsequent protein coagulation by heat, alcohol or pH adjustment, in order to impart a meat-like structure. Where an alcohol extraction is employed, the isoflavone content of the final ingredient is considerably reduced, as most isoflavones are soluble in alcohol (Wang & Murphy, 1994*a*). Conversely, extraction with water has a much smaller impact on final isoflavone levels in the final ingredient.

Over recent years, isoflavone concentrates made by solvent extraction have become available, with contents of as much as 50 % isoflavones. Their main application is in the development of foods with enhanced isoflavone levels, but with minimal impact on flavour, food quality or technological function. However, despite their significant technological advantages over soya protein isolates and concentrates as sources of phyto-oestrogens, they have found little application in European foods to date. This may be partly due to regulatory restrictions in Europe on communication of health benefits of foods to consumers. Additionally, national legislation in some countries restricts the addition of nutrients and ingredients such as isoflavone concentrates to foods.

Traditional soya foods

In the Far Eastern cultures there is along tradition of preparing foods from whole soyabeans or soya flour. These foods now form part of the European repertoire of foods and are widely available in all countries. However, beyond a relatively small subgroup of frequent consumers, the mean intake of soya-based foods is currently very low in Europe (Valsta *et al.* 2003; van Erp-Baart *et al.* 2003).

Particular varieties of soyabeans are eaten as whole beans, either cooked (normally boiled) or raw as sprouted beans. Uptake of water, and to a lesser extent leaching from the boiled food, reduces the concentration of isoflavones in these foods compared with the unprepared bean.

Traditionally prepared foods include tofu, which is a protein-rich curd made by precipitation with calcium chloride, and soya milk. Isoflavones are not lipid-soluble. However, their concentration in full-fat tofu and soya milks has been reported to be higher than in reduced-fat versions (Coward *et al.* 1998), possibly due to removal as a complex organic/polar emulsion during preparation of lower-fat versions.

Some products like miso, soya sauce and tempeh are made by fermentation using a variety of moulds, yeasts and bacteria. The fermentation phase in the preparation of these foods can be extremely prolonged, often spanning several months or years. The aglycone isomers in foods prepared in this way are the predominant forms, due to hydrolysis of the glycosides by the respective micro-organism(s) used or possibly by glucosidases in the soyabean itself (Coward *et al.* 1993; Matsuura & Obata, 1993). These differences may have implications in terms of absorption, metabolism and health in man.

The isoflavone levels in traditional soya foods largely reflect the composition of the raw material beans, but their concentrations are normally lower in prepared foods due to dilution by water used during preparation. Nevertheless, traditional soya foods are potentially a rich source of isoflavones compared with other dietary sources, such as split peas and other legumes. Table 2 illustrates the typical values that have been measured in some traditionally prepared soya foods.

Second-generation soya foods

In addition to the traditional foods described above, European consumers increasingly demand foods that not only provide health benefits, but also are in a familiar

Table 1. Isoflavone content of soya food ingredients (expressed as aglycone equivalents, wet weight basis)

Ingredient	Total isoflavones (mg/kg)	Reference
Soyabeans	560–3810	Reinli & Block (1996), Adlercreutz & Mazur (1997), Liggins et al. (2000*a,b*)
Soya flour	830–1780	Reinli & Block (1996)
Textured soya protein	701–1184	Reinli & Block (1996), Adlercreutz & Mazur (1997), Liggins *et al.* (2000*a,b*)
Soya isolates	466–615	Wang & Murphy (1994*a*)
Soya concentrates	561·1	Wang & Murphy (1994*a*)
Isoflavone concentrates	1000–400 000	Manufacturers’ data

Table 2. Isoflavone content of traditional soya foods (expressed as aglycone equivalents, wet weight basis)

Traditional food	Total isoflavones (mg/kg)	Reference
Roasted soyabeans	170–2020	USDA–Iowa State University (2002)
Miso*	260–890	Reinli & Block (1996), Adlercreutz & Mazur (1997), Liggins *et al.* (2000*a*,*b*)
Tofu	80–670	Reinli & Block (1996)
Tempeh*	69–625	USDA–Iowa State University (2002)
Natto*	460–870	USDA–Iowa State University (2002)
Dried soyabeans (boiled)	470	Liggins *et al.* (2000*a*,*b*)
Green soyabeans (boiled)	550	Franke *et al.* (1995, 1998)
Sprouted soyabeans	250–530	USDA–Iowa State University (2002)
Fermented bean curd*	390	Wang & Murphy (1994*a*)
Soya milk	13–211	Reinli & Block (1996)
Soya sauce*	13–75	Reinli & Block (1996)
Split peas (raw)	0–73	USDA–Iowa State University (2002), Liggins *et al.* (2000*a*,*b*)

USDA, United States Department of Agriculture.
* Fermented during preparation.

and possibly more palatable form. These so-called 'second-generation' soya-based foods (Wang & Murphy, 1994*a*) can be produced from whole soyabeans, flour or tofu, but may also contain soya protein concentrates or isolates. The technological and cost advantages of these ingredients make possible a wider range of foods offering potential health benefits, but without sacrificing the taste enjoyment of more familiar foods.

Such products largely take the form of analogues of Western foods, particularly dairy products including milk, yoghurts and cheeses, and analogues for meat products such as chicken, sausage, bacon and pâtés. The isoflavone content of such foods can be as high as in more traditional foods. However, they are normally produced as vegetarian alternatives to traditional foods and rarely as a source of isoflavones *per se*. Therefore, the isoflavone content of soya analogue foods varies considerably, and independently of soya content. The final content is affected by the concentration of isoflavones in the original soya component, dilution by other ingredients, such as wheat gluten, cereals or dairy components, and to a lesser extent by subsequent processing. Examples of second-generation soya foods and their isoflavone contents are given in Table 3.

Some specialist breads are made with significant levels of soya flour, linseed and other ingredients, and may be a useful source of isoflavones. However, little or no analytical data are available. One example of such breads, produced in The Netherlands, contains a soya isoflavone concentrate enabling a claim of 50 mg per 'serving'.

Soya ingredients in European foods

In addition to 'second-generation foods', soya derivatives are widely used in foods at varying concentrations for nutritional, technological and economic reasons. Examples include the use of textured soya protein as a meat extender in some commercially prepared meat products, and low levels of soya ingredients in bread to improve texture and loaf quality. The dietary significance of foods containing relatively low levels of soya phyto-oestrogens, and which are widely consumed, has been difficult to determine. This has been due partly to the unavailability of food composition tables containing sufficient values in a consistent format to allow meaningful dietary analyses of a significant number of foods. Elsewhere in this supplement, van Erp-Baart *et al.* (2003) report on the systematic compilation of national databases of soya isoflavones, carried out within the framework of the EU Concerted Action VENUS (Vegetal Estrogens in Nutrition and the Skeleton) project. Estimates for genistein and daidzein intakes, from all food sources, were derived based on national consumption data in four European countries (van Erp-Baart *et al.* 2003). This study concludes that foods with relatively low isoflavone contents provide an average of less than 0·7 mg/d,

Table 3. Isoflavone content of second-generation soya foods (expressed as aglycone equivalents, wet weight basis)

Second-generation food	Total isoflavones (mg/kg)	Reference
Meatless sausage mix (dry mix)	688–825	Manufacturers' data
Chicken bake	625–750	Manufacturers' data
Soya mince & onion	745–894	Manufacturers' data
Soya mince meat (dry mix)	533–3000	Manufacturers' data
Tofu yoghurt	164	Wang & Murphy (1994*a*)
Soya hot dog (unprepared)	150	Wang & Murphy (1994*a*)
Meatless chicken (cooked)	146	Franke *et al.* (1995)
Soya bacon	122	Wang & Murphy (1994*a*)
Soya cheese*	34–109	Manufacturers' data
Soya links (cooked)	38	Murphy *et al.* (1999)

* Fermented during preparation.

which is considered physiologically irrelevant. By contrast, the subgroup with high intakes of soya-based foods consumed on average 6–10 mg/d.

Lignans

The two primary lignans, enterodiol and enterolactone, are formed by bacterial action on lignan precursors in the intestinal tract (Setchell *et al.* 1981). The principal lignan precursor identified so far in plants is secoisolariciresinol. This is not actively oestrogenic *per se*, but biologically active metabolites, referred to as 'mammalian lignans', are formed following absorption in mammals that have been shown to possess weakly oestrogenic and anti-oestrogenic properties. Like the other phyto-oestrogens, mammalian lignans may play a role in the prevention of oestrogen-dependent diseases (Adlercreutz & Mazur, 1997; Mazur *et al.* 1998*b*; Pietinen *et al.* 2001). However, the biological effects of lignans in relation to human health have received little attention to date compared with the isoflavones.

The concentrations of precursors in foods are difficult to determine directly, but estimates have been made for several foods by an indirect analytical method, using *in vitro* fermentation to approximate the likely yield of mammalian lignans (Thompson *et al.* 1991). Concentrations of secoisolariciresinol have also been measured directly using a GC–MS method in berries (Mazur *et al.* 2000), teas and coffees (Mazur *et al.* 1998*a*).

Lignan precursors are present in a wide range of foods (Table 4), with linseed (flaxseed) showing the highest concentrations. However, linseed is a relatively minor dietary component in most European countries. The most important source of lignan precursors for most European diets is likely to be fibre-rich foods, especially the cereal brans, notably from wheat, which are consumed in relatively large amounts (Thompson *et al.* 1991). A recent study indicates that the main dietary sources of lignans in Finland are seeds, cereals, fruits, berries and vegetables (Valsta *et al.* 2003). This contrasts with the situation in the USA, where the main source is fruits (de Kleijn *et al.* 2001). Furthermore, it may be speculated that because soya isoflavones are a comparatively minor dietary component in European diets (Valsta *et al.* 2003), lignan precursors could be a more important source of phyto-oestrogens for the majority of Europeans, particularly for consumers choosing diets rich in plant-based foods.

Table 4. Lignan concentrations in common European foods (Thompson *et al.* 1991)

Food	Total lignans (mg/kg)
Flaxseed meal	675
Flaxseed flour	527
Lentil	179
Soyabean	8·6
Oat bran	6·5
Wheat bran	5·7
Kidney bean	5·6
Garlic	4·1
Asparagus	3·7
Carrot	3·5
Oats	3·4
Broccoli	2·3
Leek	2·0
Pear	1·8
Plum	1·5
Sweet potato	0·3

Acknowledgements

This review has been carried out with financial support from the Commission of the European Communities, Food and Agroindustrial Research programme CT-98-4456 'Dietary exposure to phyto-oestrogens and related compounds and effects on skeletal tissues (VENUS)'. It does not reflect its views and in no way anticipates the Commission's future policy in this area.

References

Adlercreutz H & Mazur W (1997) Phyto-oestrogens and Western diseases. *Annals of Medicine* **29**, 95–120.

Barnes S, Kirk M & Coward L (1994) Isoflavones and their conjugates in soy foods: extraction conditions and analysis by HPLC–mass spectrometry. *Journal of Agricultural and Food Chemistry* **42**, 2466–2474.

Cassidy A, Hanley B & Lamuela-Raventos RM (2000) Isoflavones, lignans and stilbenes — origins, metabolism and potential importance to human health. *Journal of Science of Food and Agriculture* **80**, 1044–1062.

Coward L, Barnes N, Setchell KDR & Barnes S (1993) Genistein and daidzein and their glycoside conjugates: anti-tumour isoflavones in soybean foods from American and Asian diets. *Journal of Agriculture and Food Chemistry* **41**, 1961–1967.

Coward L, Smith M, Kirk M & Barnes S (1998) Chemical modification of isoflavones in soy foods during cooking and processing. *American Journal of Clinic Nutrition* **68**, 1486S–1491S.

de Kleijn MJ, van der Schouw YT, Wilson PW, Adlercreutz H, Mazur W, Grobbee DE & Jacques PF (2001) Intake of dietary phytoestrogens is low in postmenopausal women in the United States: the Framingham study (1–4). *Journal of Nutrition* **131**, 1826–1832.

Farmakalidis E & Murphy PA (1985) Isolation of 6″-*O*-acetylgenistin and 6″-*O*-acetyldaidzin from toasted defatted soyflakes. *Journal of Agricultural and Food Chemistry* **33**, 385–389.

Franke AA, Custer JL, Cerna CM & Narala K (1995) Rapid analysis of dietary phytoestrogens from legumes and from human urine. *Proceedings of the Society for Experimental Biology and Medicine* **208**, 18–26.

Franke AA, Custer LJ, Wang W & Shi CY (1998) HPLC analysis of isoflavonoids and other phenolic agents from foods and from human fluids. *Proceedings of the Society for Experimental Biology and Medicine* **217**, 263–273.

Liggins J, Bluck LJC, Runswick S, Atkinson C, Coward WA & Bingham SA (2000*a*) Daidzein and genistein content in fruits and nuts. *Journal of Nutritional Biochemistry* **11**, 326–331.

Liggins J, Bluck LJC, Runswick S, Atkinson C, Coward WA & Bingham SA (2000*b*) Daidzein and genistein content in vegetables. *British Journal of Nutrition* **84**, 717–725.

Matsuura M & Obata A (1993) β-Glucosidases from soybeans hydrolyze daidzin and genistin. *Journal of Food Science* **58**, 144–147.

Mazur W & Adlercreutz H (2000) Overview of naturally occurring endocrine-active substances in the human diet in relation to human health. *Nutrition* **16**, 654–678.

Mazur WM, Duke JA, Wähälä K, Rasku S & Adlercreutz H (1998*b*) Isoflavonoids and lignans in legumes: nutritional and health aspects in humans. *Journal of Nutritional Biochemistry* **9**, 193–200.

Mazur WM, Uehara M, Wähälä K & Adlercreutz H (2000) Phyto-oestrogen content of berries, and plasma concentrations and urinary excretion of enterolactone after a single strawberry-meal in human subjects. *British Journal of Nutrition* **83**, 381–387.

Mazur WM, Wähälä K, Rasku S, Salakka A, Hase T & Adlercreutz H (1998*a*) Lignan and isoflavonoid concentrations in tea and coffee. *British Journal of Nutrition* **79**, 37–45.

Murphy PA, Song TT, Buseman G, Barva K, Beecher GR, Trainer D & Holden J (1999) Isoflavones in retail and institutional soy foods. *Journal of Agricultural and Food Chemistry* **47**, 2697–2704.

Pietinen P, Stumpf K, Männistö S, Kataja V, Uusitupa M & Adlercreutz H (2001) Serum enterolactone and risk of breast cancer. *Cancer Epidemiology, Biomarkers & Prevention* **10**, 339–344.

Reinli K & Block G (1996) Phytoestrogen content of foods — a compendium of literature values. *Nutrition and Cancer* **26**, 123–148.

Setchell KDR & Adlercreutz HA (1988) Mammalian lignans and phytoestrogens. Recent studies on their formation, metabolism and biological role in health and disease. In *Role of the Gut Flora in Toxicity and Cancer*, pp. 315–345 [IR Rowland, editor]. New York: Academic Press.

Setchell KDR, Lawson AM, Borriello SP, Harkness R, Gordon H, Morgan DML, Kirk DN, Adlercreutz H, Anderson LC & Axelson M (1981) Lignan formation in man — microbial involvement and possible roles in relation to cancer. *Lancet* **ii**, 4–7.

Thompson LU, Robb P, Serraino M & Cheung F (1991) Mammalian lignan production from various foods. *Nutrition and Cancer* **16**, 43–52.

United States Department of Agriculture–Iowa State University Isoflavones Database (2002) Database on the Isoflavone content of Foods, Release 1.3. http://www.nal.usda.gov/fnic/foodcomp/Data/isoflav/isoflav.html

Valsta LM, Kilkkinen A, Mazur W, Nurmi T, Lampi A-M, Ovaskainen M-L, Korhonen T, Adlercreutz H & Pietinen P (2003) Phyto-oestrogen database of foods and average intake in Finland. *British Journal of Nutrition* **89**, Suppl. 1, S31–S38.

Van der Schouw YT, de Kleijn MJJ, Peeters PHM & Grobbee DE (2000) Phyto-estrogens and cardiovascular disease risk. *Nutrition, Metabolism and Cardiovascular Diseases* **10**, 154–167.

Van Erp-Baart AMJ, Brants HAM, Kiely M, Mulligan A, Turrini A, Sermoneta C, Kilkkinen A & Valsta LM (2003) Isoflavone intake in four different European countries: the VENUS approach. *British Journal of Nutrition* **89**, Suppl. 1, S25–S30.

Wang H & Murphy P (1994*a*) Isoflavone content in commercial soybean. *Journal of Agriculture and Food Chemistry* **42**, 1666–1673.

Wang H & Murphy P (1994*b*) Isoflavone composition of American and Japanese soybeans in Iowa: effects of variety, crop year, and location. *Journal of Agriculture and Food Chemistry* **42**, 1674–1677.