Environmental Influences on Isoflavones and Saponins in Soybeans and Their Role in Colon Cancer\(^1,2\)

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ABSTRACT Soybeans have long been recognized as an excellent source of high-quality protein. The soybean also contains a wide variety of chemical compounds that have potent bioactivity. Among these compounds are the isoflavones and the saponins. The goal of our research was to quantify isoflavone and saponin concentrations in elite soybean cultivars grown in different environments and to identify a naturally occurring high and low variety that could be used in animal studies of colon cancer. We observed significant environment \(\times\) genotype interactions for the cultivars and selected 2 that provided the range of concentrations for isoflavones and saponins. These were grown in an adequate quantity for animal studies, which are ongoing. We explored the influence of isoflavones and saponins on human colon tumor cells in culture, Caco-2, to determine potential mechanisms through which these compounds influence the carcinogenic process. We observed the inhibition of Caco-2 cell proliferation by isoflavones and saponins, suggesting a protective effect of these compounds in colon cancer. Using purified soy saponins, we found no negative effects on mouse growth, organ weights, or intestinal morphology when the diet contained up to 3\% saponins by weight. Hence, soy isoflavones and saponins are likely to be protective of colon cancer and to be well tolerated. Continuing studies will explore the cancer-protective effects of these compounds in animal models. 


KEY WORDS: \(\bullet\) soybean \(\bullet\) colon cancer \(\bullet\) saponins \(\bullet\) isoflavones \(\bullet\) Caco-2

Soybeans contain several biologically active components that may contribute individually or synergistically to the health benefits of this plant (1,2). Among the components of soy that have been hypothesized to provide health benefits are protein, isoflavones, saponins, oils and fatty acids, fiber, and trypsin inhibitors. Soybean cultivation has generated thousands of varieties of soybeans that express distinct functional characteristics. Mostly, plants have been cultivated to optimize growth, resist diseases, and insects and maximize yield. As consumers are becoming increasingly aware of the health benefits of soy (3), attention has turned to the generation of soybeans that provide improved taste, functional characteristics, and health benefits. The goals of our research project were 2-fold: 1) To investigate the natural variation in expression of isoflavones and saponins in soybean varieties grown at different environmental locations; and 2) To determine the response of colon tumorigenesis in mice to soybeans with high or low expressions of isoflavones and saponins. In addition, we investigated the influence of soy isoflavones and saponins on cultured human colon tumor cells and the effect of dietary saponins on mouse colon mucosa.

Saponins are chemical structures consisting of triterpenoidal or steroidal aglycones with various carbohydrate moieties that are found in many plants. Soy saponins have been purified and classified by their structure into 3 groups: A, B, and E (4–7). Because of the presence of both hydrophilic and hydrophobic regions, saponins are excellent emulsifiers and foaming agents, and provide functional roles in foods. The ability of saponins to form emulsions in the intestine have lead to the investigation into their role for lowering serum cholesterol in humans. Soy primarily contains 3 types of isoflavone glycosides: genistin, daidzin, and glycitin. The isoflavone aglycones, genistein, daidzein, and glycetin, are structurally
related to 17β estradiol, hence, they are often referred to as phytoestrogens (8). In fact, soy isoflavones bind to mammalian estrogen receptors (ER) and generate estrogenic responses in vitro and in vivo. It is generally accepted that these compounds have low binding affinity for both ER alpha and beta, but preferentially bind to ER-β (9).

Little is known about the levels of isoflavones and saponins in soybean. The concentration of isoflavones in soybeans has been quantified but only on a limited scale using a few elite cultivars (10,11). Eldridge and Kwolek (12) found isoflavone concentrations in soybean to range from 1160 to 3090 μg/g. And Wang and Murphy found (13) isoflavone concentration to vary from 1176 to 3309 μg/g within a single cultivar of soybean. Hoeck et al. (10) reported that total isoflavone concentrations ranged from 1212 to 2547 μg/g. One study measured isoflavone concentrations using the same cultivars at the same locations during different years, but only 6 elite cultivars were evaluated (10). It is generally agreed that concentrations of isoflavones are highly influenced by the environment. Hoeck et al. (10) grew 6 soybean cultivars at multiple locations for 2 years and reported significant genotype × environmental interactions for concentration of nine different isoflavones. Wiebold (University of Missouri, Dept. of Agronomy, unpublished results) reported that the date of planting had a great influence on levels of isoflavones, with early planted soybean having lower concentrations of isoflavones. Tsukamoto et al. (11) discovered that high temperatures during seed development of soybeans significantly reduced concentrations of isoflavones but had no effect on levels of saponins.

Our goal was to quantify isoflavone and saponin concentrations in elite soybean cultivars grown in different environments and to identify a naturally occurring high and low variety, which could be used in animal studies of colon cancer. During the 2001 growing season, 11 soybean plant introductions and 10 elite cultivars were grown at 5 locations within Missouri: Novelty, Corning, Grand Pass, Kingdom City, and Bethel. The objective was to determine genotype × environmental interactions of isoflavone concentrations. We also wanted to determine if plant introductions behaved differently compared with the elite cultivars for isoflavone concentrations.

Significant genotype × environmental interactions were observed for the plant introductions and the elite cultivars (Table 1). The significant interactions were largely because of changes in magnitude. In addition, data shown in Table 1 indicate that, in many instances, there was a significant difference between plant introductions and cultivars in levels of isoflavones and saponins. Data in Table 2 indicate that there is generally a positive relation between concentrations of isoflavones and saponins. This was fortunate, because we wanted to identify lines from this study that could be used to construct quantitative trait loci mapping populations for total isoflavone concentrations and to increase seed for feeding studies.

Plant introduction PI 437654 had high total isoflavone (2479, 2485, 2567, 1243, and 2627 μg/g) and saponin (3999, 4024, 3951, 4886, and 5830 μg/g) concentrations at the 5 different locations. Magellan, an elite cultivar, had low total isoflavone (1288, 992, 1459, 687, and 1448 μg/g) and saponin (2209, 2402, 2797, 2364, and 2730 μg/g) concentrations at these same locations. These lines have both been increased to provide enough seed for feeding studies, which are ongoing. It is proposed that these lines be used as parents to construct 2 mapping populations for discovering quantitative trait loci associated with total isoflavone and saponin concentrations and this work is progressing.

The role of diet in colon cancer has been extensively studied since the observation by Burkitt (14) in the 1960s of a relation between dietary fiber intake and colon cancer incidence. Although evidence from epidemiological studies for a dietary factor in colon cancer exists, specific components responsible for this relation have not been identified. The World Cancer Research Fund and the American Institute for Cancer Research compiled a comprehensive review of diet–cancer relation and found convincing evidence that vegetable intake decreases the risk of colon cancer (15). Consumption of soy has been found to reduce colon cancer risk in some human population and animal studies, but the evidence is not substantial (16–18). Several case-control studies have shown reduced colon-cancer risk with increased consumption of legumes (19,20) and vegetable protein (21). There is a general consensus that diets high in red meat (and, perhaps, associated saturated fat) are strongly associated with an increased risk of colon cancer. Hence, replacing meat in the diet with foods containing soy protein may substantially reduce colon-cancer risk, both by eliminating potential carcinogenic foods and providing protective compounds. As discussed above, soy contains a complex mixture of potentially beneficial components that may function independently or synergistically to influence carcinogenesis.

Consumer interest in soy isoflavones, mainly from menopausal women, as naturally occurring estrogens has stimulated food manufacturers to create novel products containing mixtures of these compounds. The overall impact of elevated consumption of these compounds has not been thoroughly investigated. Much discussion has occurred within the scientific community regarding the safety and the efficacy of soy isoflavones relative to breast-cancer risk, with little consensus. Whereas estrogens, and potentially soy isoflavones, are

### Table 1

<table>
<thead>
<tr>
<th>Location</th>
<th>Bethel</th>
<th>Grand Pass</th>
<th>Corning</th>
<th>Kingdom City</th>
<th>Novelty</th>
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</thead>
<tbody>
<tr>
<td>Genistein</td>
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<td>**</td>
<td>NS</td>
<td>*</td>
<td>**</td>
</tr>
<tr>
<td>Glycitein</td>
<td>**</td>
<td>**</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
</tr>
<tr>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Isoflavone total</td>
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<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
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<td>NS</td>
<td>NS</td>
<td>**</td>
<td>**</td>
<td>NS</td>
</tr>
<tr>
<td>Saponin 2 and 3</td>
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<td>NS</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
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<tr>
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<td>NS</td>
<td>NS</td>
<td>**</td>
<td>**</td>
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<td>**</td>
<td>NS</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Saponin total</td>
<td>**</td>
<td>NS</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

NS, not significant.

* P < 0.05; ** P < 0.01.

### Table 2

Correlations between total isoflavone and saponin concentration at five Missouri locations in 2001

<table>
<thead>
<tr>
<th>Location</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
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<tr>
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<td>0.346**</td>
</tr>
<tr>
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<td>0.362**</td>
</tr>
<tr>
<td>Kingdom City</td>
<td>0.396**</td>
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<tr>
<td>Novelty</td>
<td>0.533*</td>
</tr>
</tbody>
</table>

NS, Not significant.

* P < 0.01; ** P < 0.05.
known promoters of breast cancer, the role of estrogen in colon cancer is less well studied. During the 1970s, colon-cancer incidence decreased in women, but not in men, aged 35–44 y (22). One possible explanation for this observation was the observed peak in fertility and the use of high-dose oral contraceptives during the prior decade. Based on these observations, either high fertility or exposure to exogenous steroid hormones could be protective of colon cancer. Corroborating this hypothesis was the conclusion from a review of the literature that the use of hormone replacement therapy by postmenopausal women was associated with a 20% decrease in colon-cancer risk (16,22).

Although there is increasing evidence that estrogen is an important mediator of colon cancer, neither the role of estrogen nor the affect of isoflavones is clear. Some animal studies have observed a protective effect of soy isoflavones on colon cancer. Reduced tumor incidence was observed in azoxymethane (AOM) treated rats fed soy protein isolate containing isoflavones (23). And, reduced aberrant crypt foci, purported precancer lesions, were observed in AOM-treated rats fed soy flakes or soy flour containing isoflavone compared with soy concentrate without isoflavone (24). We recently reported that mice fed diets containing soy protein with estrone, a naturally occurring mammalian estrone, were protected from colon-carcinogenesis (25). In our study however, genistein (250 mg/kg) added to a soy protein diet did not reduce colon-tumor incidence. A previous report also found genistein added to a casein diet had no effect on tumorigenesis (26). More aberrant crypt foci were observed in AOM-treated rats fed soy protein with isoflavone compared with soy protein without isoflavone, but no differences in tumor incidence was observed (27). And no difference in tumorigenesis was reported in ApcMin mice fed low-isoflavone or isoflavone-rich soy protein (28). In our study (25), soy protein independently reduced colon-tumor multiplicity and burden compared with the casein control diet. Hence, some of the discrepancies among animal studies may be caused by confounding bioactive components of the diet, such as the protein source, saponins, soy fiber, or other factors. These bioactive components likely have a significant influence on the ability of isoflavones to affect carcinogenesis in these animal models and will impact the response of human cancers. Therefore, it is critical that whole soy food complexes be examined for their ability to influence colon-cancer risk.

To determine the ability of isoflavones to affect colon tumorigenesis, we incubated human colon tumor cells, Caco-2, with genistein, daidzein, or soy extract enriched in isoflavones (Novasoy; Archer Daniels Midland). Genistein was most effective in inhibiting cell growth, but both daidzein and Novasoy also reduced proliferation (Fig. 1). We investigated the effect of purified soy saponins on cultured human colon tumor cells, Caco-2. The Group A saponins were effective in inhibiting cell proliferation at 20 μg/mL, but only when provided in the aglycone form (Fig. 2). A similar inhibitory effect of soy saponins on HCT-15 cells was previously observed (29), however damage to the cell membrane occurred with these preparations (30). With our purified saponin fractions, no negative effects on cell morphology were observed.

To define a maximal toxic dose in mice, we fed young, C57blackJ mice from our colony diets containing purified soy saponin glycosides. Four separate experiments were performed with increasing doses of saponins. In each experiment, groups of 10 mice were fed AIN93G-based diets for 4 wk. The diets
for experiment 1 contained 0, 250, 500, or 1000; experiment 2, 0, 100, or 2000; experiment 3, 0, or 5000; and experiment 4, 0, 1000, or 2000; experiment 5, 0, and 2000; experiment 6, 0, 250, 500, or 1000; experiment 7, 0, or 5000; and experiment 8, 0, 250, 500, or 1000 mg saponins per kg. Saponins were prepared by Dr. Mark Berhow. Each group contained 10 mice that were fed the diets for 4 wk. Data are means. No significant differences due to treatment were observed.

In conclusion, we have identified 2 soybean varieties that possess distinct qualities of isoflavones and saponins that will be fed to mice to determine their ability to prevent colon cancer. From in vitro work, we have observed interactions between soy protein and isoflavones that suggest soybean foods will differ from purified extracts of isoflavones and, perhaps saponins, in their anticancer effects. Caution should be used to extrapolate research using purified isoflavones or saponins to whole soy foods as interactions among bioactive components likely affect biological response. Soy saponins may be important anticancer compounds present in soy due to their ability to inhibit tumor cell growth without altering normal colon morphology.

LITERATURE CITED