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Organic farming research project report submitted to the Organic Farming Research Foundation:

Project Title:

Phytoestrogen content of conventionally and organically grown soybeans

FINAL PROJECT REPORT

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Introduction:

Soy-based foods have been the subject of recent research due to increasing evidence that soy phytoestrogens may modify the pathogenesis of some hormone-dependent and hormone-independent diseases'. Studies have shown the isoflavones genistin and daidzin may have beneficial effects on menopausal symptoms', osteoporosis[3], and coronary heart disease [4-5]. These findings have led to clinical recommendations of greater dietary intake of phytoestrogen-containing soy products. Other reports suggest there may be adverse effects from over-ingestion of phytoestrogens, especially in infants[6,',s]. This increase in phytoestrogen ingestion for medical purposes coupled with the inconsistency of how phytoestrogens may effect the human body make it important that consumers and clinicians know the amount and kind of phytoestrogens present in soy-based products.

Studies show that the level of phytoestrogens in soybeans may vary significantly. Environmental factors such as temperature, precipitation, humidity, wind, relative soil conditions, slope of terrain and irrigation may effect the concentration of phytoestrogens by as much as 300 percent". Farming methods may therefore be a critical factor in phytoestrogen content.

A recent study shows significant reductions in phytoestrogen content between genetically modified Roundup Ready soybeans and conventional soybeans more heavily treated herbicides". However no study has examined how pesticide free organic and conventional farming methods affect the phytoestrogen content of genetically identical beans.

Materials and Methods:

To quantify the levels of biologically active phytoestrogens present in conventional versus organic soybeans, we tested three varieties of soybeans supplied by the Rodale Institute Experimental Farm in Kutztown, Pennsylvania: 'Vinton 81,' `Iowa 3001' and `HP 204.' These varieties are all food grade soybeans, specifically developed for use in soyfoods such as tofu, soymilk, and soynuts. In such products, the soybeans are commonly sold as whole, unsplit beans with a protein content of greater than 40 percent and a large size (less than 200 beans per pound).

The three varieties were grown in paired plots with similar soil profiles on the same farm during the 1998 second growing season. One plot was farmed organically while the other was farmed conventionally. The three conventionally grown soybean varieties were grown using a modified no-till system which included the use of the herbicides Roundup, Pinnacle and Poast for weed control. No other pesticides were used. Organically grown soybeans were sown and grown concurrently with the conventional varieties but without the use of chemicals. After harvest, the seeds were stored at approximately 40° F for up to one year before analysis.

Blinded samples of ten seeds of each variety were analyzed in matched pairs containing

either conventional or organic seed samples. In both instances, refrigerated seeds were pulverized in a stainless steel-bladed grinder and immediately extracted with cold 80% methanol. After separation by reverse-phase, high-pressure liquid chromatography, isoflavones were isolated and measured in duplicate against an internal standard by absorption at 260 rim".

Results:

Table I shows the phytoestrogen content of conventional and organic soybeans. In the HP204 and Iowa varieties, the total levels of isoflavones present in the organic sample were comparable to those in the matched conventional sample. In the Vinton variety, the total isoflavones in the organic sample were slightly higher than the matched conventional variety. However, this difference was not statistically significant and was largely attributable to a single high value for glycitin in one but not the other organic sample.

We also tested for the individual contributions of key biologically active isoflavones daidzin, genestin and glycitin to the overall phytoestrogen levels. The three isoflavones were analyzed in different molecular forms: the (3-glycosides (daidzin, glycitin and genistin), the malonylglycosides (malonyl-daidzin, malonyl-glycitin and malonyl-genistin) and the aglycones (daidzein and genestein). The aglycones were in quantities too low to be measured precisely (<_7 gg/g) and hence were excluded from analysis of the results.

As shown in Table 2, daidzin and genestin were present in roughly equal proportions and together made up approximately 85 percent of the total phytoestrogens. There were no evident differences in the patterns of the contribution made by individual isoflavones to the total level between conventional and organic varieties. Although the average glycitin contribution from the organic Vinton 81 variety appeared to be twice that of the matched conventional beans, this result was attributable to the single large glycitin value (319 µg/g) from one of the organic Vinton samples.

In previous studies done by our group, phytoestrogen content ranged from approximately 1200 - 2000 micrograms per gram'. These values are approximately twice the 600-1000 µg/g range seen in the present organic versus conventional comparison. However, as shown in Table 3, when the results are calculated per bean rather than by concentration, the findings are comparable.

Figure l provides a graphical depiction of the relative amounts of each of the major phytoestrogens to the composition of each soybean variety. Minor differences in the proportions of phytoestrogens by variety are evident, such as the greater amount of daidzin in the Vinton variety.

Discussion:

Our data suggest conventionally and organically grown soybeans of the same variety have

comparable levels of phytoestrogens. In keeping with our previous studies, we observed subtle differences in phytoestrogen makeup by variety but little betweensample variability. Altering the growing conditions by changing pesticide regimes appeared to have little or no influence on phytoestrogen content. This lack of variability between organic and conventional soybeans suggests that herbicide treatment or nontreatment of genetically similar soybeans does not change the plant's phytoestrogen development. This observation is relevant to our previous study which showed significant phytoestrogen variability between herbicide-sprayed conventional and genetically modified soybeans[14]. One explanation of these findings, that differing levels of herbicide use between the conventional and genetically modified beans affected the phytoestrogen development differently, is not supported by the present study. Our present findings reinforce the conclusion that the differences reported in Roundup Ready soybeans were likely a result of the genetic modification process and not differential herbicide use.

Our findings may also be relevant to the general consumer of soy-based food products. Our study shows that organic and conventional soybeans have comparable levels of phytoestrogens. We cannot exclude the possibility that other nutritional components in organic and conventional soybeans differ. However, our data suggest consumers who may wish to reduce their ingestion of pesticides by eating organic soybeans will not lose out on phytoestrogens by making such a choice.

Although sample-to-sample variability showed no statistically significant differences between the organic and conventionally grown varieties, we observed enough variability within the organic samples to warrant further testing. The 350% difference in glycitin levels between the two Vinton organic bean samples may be of importance (see Table 1). The magnitude of this variability may or may not be of clinical or dietary significance. However, for those individuals who ingest phytoestrogens to mediate the effects of hormonal activity associated with menopause or other related effects of normal physiology, it would be of importance to accurately know the quantity and type of phytoestrogens present in specific soybean varieties. In particular, infant consumption of soy-based formulas with extreme variability of isoflavone content may be of clinical significance [15,16]. Further research is needed to determine if organic Vinton or other soybean varieties show reproducible variation in this or other key phytoestrogens.

The discrepancies apparent in phytoestrogen concentration between current and a past study can be rectified. In contrast to the soybeans used for this study which were large and had a 40 percent protein content, the soybeans used in the previously cited study were developed for seed and were only about half as heavy per bean. The apparent concentration differences are resolved when the data is adjusted for bean weight (see Table 3). We recommend that future publications involving soybean phytoestrogen content report findings in micrograms per bean as well as

concentration per gram to avoid apparent discrepancies.

Overall, our findings of equivalence between chemically treated and untreated soybeans do not support the hypothesis of Eldridge and Kwolek that herbicide spraying will increase phytoestrogen levels". The organic and conventionally grown soybeans in our tests had remarkably similar profiles and concentrations of phytoestrogens suggesting that organic farming methods do not alter this important dietary ingredient of soybeans.

References:

1Murkies, A.L. , Wilcox, G., and Davis, S. (1998). Phytoestrogens. *Journal of Clinical Endocrinol Metal;* 83, 297-303.

2 Belchetz, P.E. (1994) Hormonal Treatment of Postmenopausal Women. *New England Journal of Medicine.* 330; 1062-1071.

3 The Writing Group for the PEPI Trial. (1996) Effects of Hormone Therapy on Bone Mineral Density. *JAMA.* 276;1389-1396.

4 Grodstein, F., Stamfer, M.J., Manson, J.E., et al. (1996)Postmenopausal Estrogen and Progesterone use and Risk of Cariovascular Disease. *New England Journal of Medicine.* 335; 453-461.

5 Tikkanen, M.J., Wahala, K., Ojala, S., Vihma, V., Aldercreutz, H. (1998) Effect of Soybean Phytoestrogen on Low Density Lipoprotein Oxidation Resistance. *Proceedings of the National Academy of Sciences* 95;3106-3110.

6 Setchell, K.D.R., Zimmer-Nechamias, L., Cai, J., and Heubi, J.E. (1997). Exposure of Infants to Phytoestrogens from Soy-based Formula. *Lancet 350,* 23-27.

7 Robertson, I.G.C. (1995). Phytoestrogens: Toxicity and Regulatory Recommendations. *Proc Nutr Soc* NZ 20, 35-42.

8 Irvine, C., Fitzpatrick, M., Robertson, I., and Woodhams, D. (1995). The Potential Adverse Effects of Soybean Isoflavones in Infant Feeding. *NZ Med J* ***108,*** 218

9 Eldridge, A.C. and Kwolek, W.F. (1983) Soybean isoflavones . Effect of Environment and Variety on Composition. *Journal of Agriculture and Food Chemistry.* 31; 394-396.

10 Choi, J.-S., C. Kwon, T.-W., and Kim, J.S. (1996) Isoflavone Contents in some Varieties of Soybeans. *Food and Biotechnology 5;* 167-169.

11Lappe, M.A., **E.B.** Bailey, C. Childress, and K.D.R. Setchell. (1999). Alterations in Clinically Important Phytoestrogens in Genetically Modified, Herbicide-Tolerant Soybeans. *Journal of Medicinal Food 1, 4*. 12

12 Methods previously described in Setchell et al. 1997 (see reference 6).

13Lappe, M.A., E.B. Bailey, C. Childress, and K.D.R. Setchell. (1999). Alterations in Clinically Important Phytoestrogens in Genetically Modified, Herbicide-Tolerant Soybeans. *Journal of Medicinal Food 1, 4*.

14 Lappe, M.A., E.B. Bailey, C. Childress, and K.D.R. Setchell. (1999). Alterations in Clinically Important Phytoestrogens in Genetically Modified, Herbicide-Tolerant Soybeans. *Journal of Medicinal Food 1, 4*.

15 Robertson, I.G.C. (1995). Phytoestrogens: Toxicity and Regulatory Recommendations. *Proc Nutr Soc* NZ 20, 35-42."

16 Irvine, C., Fitzpatrick, M., Robertson, I., and Woodhams, D. (1995). The Potential Adverse Effects of Soybean Isoflavones in Infant Feeding. *NZ Med J* ***108,*** 218.

17 Eldridge, A.C. and Kwolek, W.F. (1983) Soybean isoflavones . Effect of Environment and Variety on Composition. *Journal of Agriculture and Food Chemistry.* 31; 394-396.

Table 1. **Phytoestrogen content of conventional and organic soybeans**[†] (µg/gram)

	β-Glycosides and Malonylglycosides			Aglycones			
Seed Variety Sample	*Daidzin*	*Genistin*	*Glycitin*	*Daidzein*	*Genestein*	Total	Mean (Confidence Interval)
HP204 A	312	368	122	7	5	814	**825 (808.5 - 841.5)**
HP204 B	329	377	120	7	5	836	
HP204 A	275	271	108	4	3	663	**747 (627.5 - 866.5)**
HP204 B	348	339	136	5	4	831	
Iowa 3001 A	304	283	134	0	0	722	**757 (704 - 810)**
Iowa 3001 B	340	318	128	5	0	791	
Iowa 3001 A	346	451	121	6	6	928	**796 (613 - 979)**
Iowa 3001 B	258	314	83	5	4	663	
Vinton 81 A	300	333	87	0	0	720	**787 (692.6 - 881.4)**
Vinton 81 B	477	298	79	0	0	854	
Vinton 81 A	334	376	319	5	5	1038	**949 (825.6 - 1072.4)**
Vinton 81 B	351	409	91	6	5	861	

[†] Organic seed data presented in shaded rows; ten beans per value.

Table 2. **Relative Contributions of Isoflavones in Organic and Conventional Soybeans**[†]

Seed Variety	Total Isoflavones (µg/gram)	Daidzin contribution	Genistin Contribution	Glycitin contribution
HP204	812	46 %	39 %	15 %
HP204	738.5	41 %	42 %	17 %
Iowa 3001	753.5	40 %	43 %	17 %
Iowa 3001	786.5	38 %	49 %	13 %
Vinton 81	787	40 %	49 %	11 %
Vinton 81	940	42 %	36 %	22 %

[†] Organic seed data presented in shaded rows.

Table 3. **Comparison of Isoflavone Levels: Concentration Versus per-Bean Composition**[†]

Seed Variety	Total Isoflavone Concentration[*] (µg/gram)	Single Bean Weight (grams)	Total Isoflavones per Bean (micrograms)
H5545 B[1] (conv)	2,015.7	.131	**264.06**
H5545 C[1] (conv)	1,718.4	.143	**245.73**
Vinton 81 (conv)	787	.247	**194.39**
Vinton 81 (org.)	949	.252	**239.15**

[†] Organic seed data presented in shaded rows.
[*] Average of ten beans.
[1] Values taken from Lappé et al. 1999.

Figure 1. **Average Isoflavone Levels in Conventional and Organic Soybeans**