Note on Coumestrol in Soybeans and Fractions at Various Germination Times[1]

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ABSTRACT

Coumestrol, an estrogenic compound found in most forage plants, was determined by high performance liquid chromatography in germinated and ungerminated (Amsoy 71, Clark 63, and Columbus variety) soybeans and fractions therefrom. The coumestrol increased with increasing germination time and the increase in concentration of coumestrol ranged between eightfold and 200-fold depending on variety and germination time. Coumestrol level was highest in the hulls, which are readily separable from the beans.

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During germination of soybeans, the content of protein, amino acid, and vitamins (Shurtleff and Aoyagi 1975) and the concentration of the estrogen, coumestrol, (Wada and Yuhara 1964) increase. Germinated beans contain 70–150 times more coumestrol than do ungerminated beans. Lookhart et al (1978, 1979) published a method for quantitating coumestrol in soybeans by high performance liquid chromatography. They determined the concentration of coumestrol in ungerminated Clark 63 variety whole beans, hulls, and endosperms plus germs to be 0.05, 0.20, and 0.05 μg/g, respectively. Because soybeans are used in food and feed additives, the location of this estrogen in the germinated bean should be determined. This note presents data that suggests that the coumestrol concentration can be minimized by varietal selection and fractionation to eliminate the highest coumestrol fractions.

MATERIALS AND METHODS

Chemicals and Reagents

Water was distilled and deionized. All extraction solvents were analytical reagent grade; all other solvents were of high purity "distilled in glass" grade, purchased from Burdick and Jackson Laboratories, Inc., Muskegon, MI. Coumestrol from Eastman Organic Chemicals was used without further purifications because thin-layer chromatography showed only one spot. Clark 63 and Columbus variety soybeans (1977 crop) were supplied by Carl B. Overley, associate professor, Agronomy Department, Kansas State University. Amsoy 71 variety soybeans (1977 crop) were supplied by Illinois Foundation Seeds, Champaign, IL.

Germination of Soybeans

Clark 63 and Columbus varieties were sprouted by placing 100 beans on several layers of water-saturated filter paper in a glass enclosure at 20 ± 2°C. Swelling was complete in 3 hr and germination was 96% in 24 hr. Eighty seeds of each variety germinated for 48 hr were planted 1 in. deep in a 1:1 mixture of potting soil/vermiculite and watered. The acrospires (rootlets) at that time were about 1 in. long, and after seven days the roots were 2–4 in. long and the epicotyls (top growth or plumules) 2 in. long.

The germination of Amsoy 71 variety was optimized as follows: Soybeans were steeped 12 hr in quart-sized brass screen baskets suspended in a vigorously stirred and aerated Warburg water bath at 23.5 ± 0.1° C. The bath water was changed at 2 and 6 hr. At 12 hr the beans had reached 60% hydration, were rinsed with distilled water, were then wrapped in wet paper towels, and incubated at 23.5 ± 0.5° C, 95–100% relative humidity on brass screen trays.

Fractionation of Germinated Soybeans

Germinated soybeans were manually fractionated into hulls (seed coats), cotyledons (endosperm) plus germs, epicotyls (plumules), hypocotyls less roots (stems), and roots, as determined by visual inspection. The ungerminated beans were separated mechanically as described by Lookhart et al (1978). The Amsoy 71 fractions not used immediately were ground in a Stein mill for 1 min with an equal weight of water, frozen in large bottles in thin shells, and lyophilized.

Extraction

The extraction and analysis of germinated and control soybean fractions followed published procedures (Lookhart et al 1978).

RESULTS AND DISCUSSION

The results, the averages of triplicate analyses of duplicate samples on Clark 63, Columbus, and Amsoy 71 varieties (1977 crop) germinated for various times, appear in Table I. For Clark 63 whole beans, coumestrol concentration at zero germination time (0.05 ppm) and coumestrol concentrations of the fractions were identical with published levels (Lookhart et al 1978) and consistent with the concentration in analogous fractions of the other varieties.

Of the varieties tested, Amsoy 71 ungerminated whole soybeans had the lowest coumestrol concentration, 0.02 ppm. Apparently coumestrol levels differ among soybean varieties as has been shown for clovers (Bickoff et al 1958, Guggolz et al 1961) and alfalfa (Hanson et al 1965).

The coumestrol level for Columbus variety whole beans germinated two days was lower than for Clark 63 variety beans germinated two days. At day 2 of germination, the coumestrol level of the Columbus variety was lower than the level at 0.5 day germination of the Amsoy variety, and because coumestrol concentration increases on germination, the level at two days' germination was lowest for the Columbus variety. A seven-day germination study of the Columbus variety was made to obtain sufficient quantities of hypocotyls less roots (stems) and epicotyls for determination of their coumestrol concentrations and to determine the effects on this variety of longer germination times. After seven days of germination there was little, if any, coumestrol in the epicotyls or hypocotyls less roots, which opposes findings (Loper and Hanson 1964, Hanson et al 1965) that in alfalfa the leaves contain the largest amount. Columbus variety was the most resistant to increases in coumestrol concentration; at seven days' germination, the whole beans contained less coumestrol than Clark

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Mention of specific instruments or trade names is made for identification purposes only and does not imply any endorsement by the U.S. government.

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TABLE I
Coumestrol Content of Clark 63, Columbus, and Amsoy 71 Soybeans and Fractions, 1977 crop[a]

Soybeans and Fractions	Coumestrol (ppm)							
	Clark 63 Germination Time (days)		Columbus Germination Time (days)			Amsoy 71 Germination Time (days)		
	0	2	0	2	7	0	0.5	3
Whole beans	0.05	1.3	0.09	0.23	0.75	0.02	0.30	3.94
Hulls	0.20 (33)[b]	11.0	0.24 (41)	0.53	2.1 (44)	0.20 (58)	NA	15.21 (54)
Cotyledons plus germ	0.05 (67)	Trace	0.06 (59)	0.18	0.42 (46)	0.02 (42)	NA	1.35 (37)
Epicotyl		NA[c]		NA	ND[d]			
Hypocotyl less roots		NA		NA	ND		NA	
Roots		ND		0.21	0.96 (10)		NA	7.90(9)[e]

[a]All analyses are averages of at least three replications of duplicate samples.
[b]Values in parentheses are weight percent of the total coumestrol.
[c]NA = Fraction not available.
[d]ND = Not detectable by this method.
[e]Total hypocotyl.

at two days or Amsoy at three days' germination.

The coumestrol concentration for the three-day germinated Amsoy 71 variety was much higher than for the two-day Clark 63 or seven-day Columbus varieties, especially in the root fractions.

Table I shows two major trends: Coumestrol concentration was higher in hulls than in any other fraction at each germination time and increased with germination time except for the cotyledons of Clark 63. The high levels in the hulls were probably related to some external microbial source as has been reported (Loper and Hanson 1964, Hanson et al 1965, Bickoff et al 1967, 1969). Because the hulls made up only 11–15% of the mass of the bean, the high levels did not greatly affect the whole bean coumestrol concentration.

The weight percentage of the total coumestrol in each fraction was calculated as the coumestrol concentration (in ppm) of a particular fraction multiplied by that fraction's percentage of the total bean weight, divided by the sum of the products of coumestrol concentration and weight percentage for each fraction of the bean. These values, listed in parentheses in Table I, give in relative terms the fractions of total coumestrol in each fraction. Therefore, removal of any fraction would reduce the total coumestrol in the remainder of the bean by an amount equal to the weight percentage of the removed fraction. Thus, the removal of the hulls (Lookhart et al 1978) would reduce the amount of coumestrol by 33–58%, depending on variety and germination time. In the ungerminated beans, 42–67% of the coumestrol is in the cotyledons, which comprise about 89% of the mass of the bean, whereas in germinated beans 37–46% of the coumestrol is in the cotyledons. Therefore, dehulling germinated beans would reduce the total coumestrol level by 54–63%.

The eightfold to 197-fold increase in coumestrol concentration in whole beans with germination time (Table I) confirms the 70- to 150-fold increase reported (Knuckles et al 1976). Probable reasons for the wide range in increases are germination time and inherent varietal differences in coumestrol production. Those differences could be related to resistance to attack by unknown, airborne organisms as reported by researchers (Loper and Hanson 1964; Hanson et al 1965; Bickoff et al 1967, 1969) who related increases in coumestrol concentration to microbial invasion.

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