Factors Affecting the Concentration of a Nutraceutical Lignan in Flaxseed

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Abstract

There is considerable interest in the use of naturally occurring plant components to prevent or treat a variety of human diseases. One such phytochemical, secoisolariciresinol diglucoside (SDG), can be isolated and purified from flax (*Linum usitatissimum*). SDG has been shown to have potential therapeutic benefits in some hormone dependent cancers, heart disease, diabetes, and even in some auto-immune diseases such as lupus nephritis. SDG is bio-synthesized from the amino acid phenylalanine and accumulates in the mature flaxseed as part of a larger chemical complex. As many as 12 varieties of flaxseed grown at locations in both Manitoba and Saskatchewan for up to 11 years have been analyzed for SDG content. Both variety and year had significant effect of SDG concentration in defatted flaxseed meal. Studies of shorter duration and with fewer varieties where soil nitrogen (N), phosphorus (P), sulfur (S) and/or potassium (K) levels were supplemented have also been conducted. In general, nutrient supplementation had little effect on SDG concentration. Where N boosted flax yield, reductions in SDG were observed, but much smaller proportionally than the increase in yield.

Introduction

Flax has long been grown as a source of linen fiber and linseed oil. In the last decade there has been a great deal of research on the seed component called SDG. This phytochemical is but one example of a larger group of compounds referred to as lignans. SDG has been shown to have potential benefits in some forms of cancer, heart disease, lupus nephritis, and diabetes (Clark and Ogborn, 2003; Prasad, 2000, 2001, 2002; 2003, et al., 2000; Rickard-Bon and Thompson, 2003).

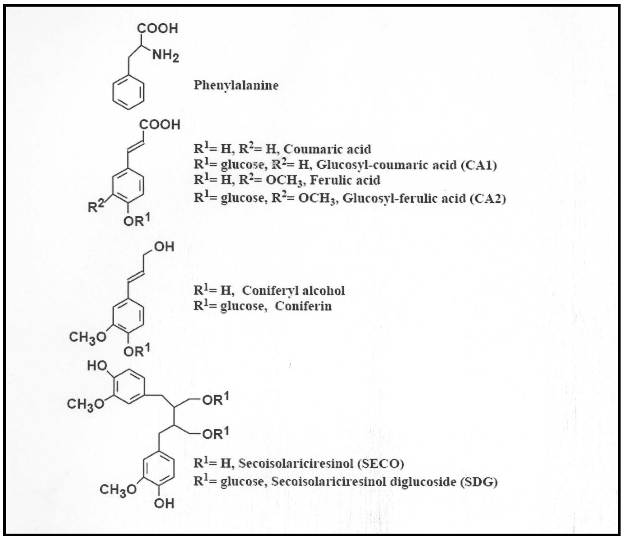

Figure 1. Chemical structure of flax lignans.

The biosynthesis of some lignans, including secoisolariciresinol, the aglycone of SDG, have been extensively studied (Ford et al., 2001; Xia et al., 2000). The pathway is summarized in **Figure 1**. As illustrated, the amino acid phenylalanine is converted into cinnamic acid that undergoes ring oxidation to coumaric acid and then both oxidation and methylation to ferulic acid. Ferulic acid is reduced into the corresponding alcohol, coniferyl alcohol. Two coniferyl alcohols are enzymatically oxidized and dimerized followed by some additional enzymatically controlled rearrangements and glucosylation which forms SDG. In flax seed, SDG is not found free but is incorporated into a larger complex (Ford et al., 2001; Kamal-Eldin et al., 2001). Careful chemical

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degradation of this complex also yields the cinnamic acid derivatives, glucosyl-coumaric acid (CA1) and glucosyl-ferulic acid (CA2).

It was demonstrated by Muir et al. (2000) that coniferin, the storage form of coniferyl alcohol, was at maximum concentration at about 5 days after start of flowering. Coniferyl alcohol reached maximum concentration a few days later while SDG reached maximum concentration in the seed by about 20 days after start of flowering.

Based on chemical analysis of eight varieties of flax seed that were collected from seed grown at four locations in 4 consecutive years, it was reported previously that flax seed meal contains between 9 and 30 mg of SDG per gram of defatted meal. The main source of variation noted from this sample set was year with secondary effect due to variety. The influence of location was of lesser significance (Westcott and Muir, 1996). It is known that CA1 and CA2 concentrations also vary with variety. Vimy has a very low combined concentration of the cinnamic acids, 1.3 mg/g meal, while other varieties contain between 8 to 16 mg/g defatted meal (Unpublished, Westcott and Muir).

In the above sample set, soil nutrients were not specifically measured or adjusted. It was of interest therefore to examine the effects of added soil nutrients on the concentration of SDG, CA1, and CA2. Seed was obtained from previously conducted fertility research studies that had been designed to measure other agronomic indicators.

1. Phosphorus study. The study was conducted in 1995 and 1997 using the variety AC McDuff. The 10 and 30 kg P_2O_5/ha rates were applied only in 1997. The least square means (presented) adjust for the effect of year on those two treatments, since the 1997 crop had lower SDG and yield levels than the 1995 crop. The yield was weakly correlated to CA1 and CA2 ($r=0.27$; $p<0.10$). Intermediate rates of P resulted in lower SDG levels than either zero or higher rates, while yield did not respond to P in these soils (**Table 1**).

Table 1. SDG, CA1, or CA2 concentrations and yield in response to applied P.

P_2O_5 applied, kg/ha	SDG, mg/g	CA1, mg/g	CA2, mg/g	Yield, kg/ha
0	11.8	0.94	1.41	2330
10	11.1	0.90	1.37	2634
20	11.4	0.89	1.32	2396
30	11.3	0.91	1.32	2368
40	12.0	0.95	1.42	2398
Contrasts:				
linear p	NS	NS	NS	NS
quadratic p	0.04	0.06	0.05	NS

2. Nitrogen by P study. Conducted 1996 and 1998, this was a full factorial design with four rates of each of N and P. Interactions between N and P were not significant (NS; $p>0.05$) for all four attributes (**Table 2**). The yield increased with N rate, but at a diminishing rate, as indicated by significant linear and quadratic components. The concentrations of SDG and the cinnamic acids decreased linearly with increasing N rate. The level of P had no effect, except that intermediate rates of P produced the highest levels of CA2 and the lowest yields.

Table 2. SDG, CA1, or CA2 concentrations and yield in response to applied P and N.

N rate, kg/ha	SDG, mg/g	CA1, mg/g	CA2, mg/g	Yield, kg/ha
0	17.6	1.74	1.17	1579
40	17.5	1.73	1.13	1874
80	17.4	1.63	1.01	2027
120	17.0	1.57	1.03	2097
Contrasts:				
linear p	0.024	<0.0001	<0.0001	<0.0001
quadratic p	NS	NS	NS	0.0007
P_2O_5 rate, kg/ha				
0	17.3	1.64	1.09	1945
15	17.5	1.71	1.10	1905
30	17.4	1.67	1.12	1819
45	17.2	1.67	1.03	1909
Contrasts:				
linear p	NS	NS	NS	NS
quadratic p	NS	NS	0.029	0.044

3. Cultivar by N study. The study was conducted in 1997 to compare three cultivars at four N rates. The yield increased with increasing N but with a diminishing response at higher rates (**Table 3a**). The glucosyl-ferulic acid (CA2) increased with increasing N up to 60 kg/ha However, SDG and glucosyl-coumaric acid (CA1) were not affected by N rate.

The Flanders cultivar had a modest increase in SDG as N increased, while the other two decreased (**Table 3b**). This was the only cultivar-N interaction with statistical significance at $p<0.01$. The difference in response between the low-SDG cultivar and the two that were generally higher in SDG is interesting.

Table 3a. SDG, CA1, or CA2 concentrations and yield in response to applied N.

N rate, kg/ha	SDG, mg/g	CA1, mg/g	CA2, mg/g	Yield, kg/ha
0	17.6	1.74	1.17	1579
0	13.5	1.8	1.5	1675
30	13.2	1.8	1.6	1991
60	13.6	1.8	1.7	2039
90	12.9	1.8	1.6	2014
Contrasts:				
linear p	NS	NS	0.033	0.0019
quadratic p	NS	NS	NS	0.021

Table 3b. SDG in mg/g as affected by cultivar and N rate.

N rate, kg/ha	AC Emerson	AC McDuff	Flanders
0	16.4	13.4	10.6
30	16.2	13.4	10.1
60	16.1	12.3	12.4
90	14.7	12.4	11.6

4. NPKS study. This study was conducted 1996-1998 in Indian Head and 1996-1997 in Lemberg for a total of 5 site-years. Seed yields were significantly increased only by N (**Table 4**). Nitrogen significantly decreased the concentration of CA1 and CA2, but the decrease in SDG was only marginally significant. The yields were increased by a greater proportion (36%) than SDG, CA1, or CA2 were reduced (10%-14%). It should be noted that in 1998 at Indian Head, yields were highest of all 5 site-years but there was no effect of fertility treatment on any of the phytochemical attributes.

Table 4. SDG, CA1, and CA2 concentration and yield in response to applied NPKS fertilizers.

Treatment	SDG, mg/g	CA1, mg/g	CA2, mg/g	Yield, kg/ha
Check	15.8	1.48	0.99	985
N	15.2	1.28	0.88	1340
NP	14.8	1.27	0.85	1440
NPK	15.1	1.31	0.86	1391
NPS	15.1	1.39	0.88	1422
NPKS	14.9	1.28	0.83	1476
Contrasts:				
N (N-check)	0.11	<0.0001	0.0001	<0.0001
P (NP-N)	NS	NS	NS	NS
K (NPK+NPKS-NP-NPS)	NS	NS	NS	NS
S (NPS+NPKS-NP-NPK)	NS	NS	NS	NS

Conclusions

Added nutrients applied at time of seeding were not effective in increasing the concentration of SDG in flax seed. It is possible that application of added nutrients nearer flowering time may cause an increase in SDG concentration. These studies confirm earlier observations that there is significant variability in the SDG concentration in registered cultivars of flax seed. Thus, it might be possible to increase SDG concentrations using conventional breeding strategies.

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