GENETIC AND ENVIRONMENTAL MODIFICATION OF THE CHLOROGENIC ACID CONTENT OF SUNFLOWER SEEDS

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Sunflower seeds are a promising source of edible protein. However, the phenolic compound, chlorogenic, caffeic and quinic acids present in sunflower seeds seriously discolor the protein during alkali extraction of the meal (5).

Phenolics are present in all sunflower species and occur throughout the plant. Within the seed the largest portion of phenolics are in the cotyledon with very low levels in the pericarp (4). The seeds near the edge of the head have higher levels than those in the middle (8). This probably reflects relative maturity. Prolonged storage of sunflower seed reduced the amount of chlorogenic acid but increased the level of quinic acid (8). Drying at elevated temperatures accelerated this process (6).

Environmental factors also influence the development of phenolic compounds. In leaves the phenolic content increases with maturity then declines with senescence (3). The effects of stress are variable. In cotton leaves the level of phenolics declines with either nutrient or moisture stress (1), whereas in sunflowers the reverse was reported (3).

Many techniques and processing methods have been devised to extract phenolics from seeds in order to produce the desired white protein isolate. These methods include alkali extraction under inert gas, followed by membrance filtration (7), prewashing with 95% ethanol (8) and the use of antioxidants (2). Unfortunately most methods suffer from a combination of incomplete extraction of phenolics, loss of protein, expensive reagents and pollution.

If protein is to be economically produced, either improved methods of extracting phenolics must be found or the chlorogenic acid content of sunflower seeds consilerably reduced to make existing extraction techniques more efficient.

This report outlines studies to reduce the natural levels of chlorogenic acid in sunflower seeds. They include the influence of growing conditions, genotype-environment interactions, the pattern of chlorogenic

acid synthesis and a search for genetic variability. All seed samples used in these studies were dehulled, ground, extracted with petroleum ether and dried. The meal was then extracted with 80% ethanol at pH 4.5 by refluxing for 30 minutes. Aliquots were read at 323 nm on a recording spectrophotometer.

RESULTS AND DISCUSSION

Growing location. Growing location had a significant effect on the chlorogenic acid content of seeds. In three years of tests with seven varieties it was found that within relatively similar environments, for example, three locations within 115 km of each other in the sunflower growing area of Manitoba, locations were significantly different in only one of the three years. However, when the test locations were separated by up to 1100 km and ranged in elevation from 300 to 900 m, the effects were significant each year. In 1973 the location averages ranged from 2.6 to 4.3%. It appears that rainfall influenced the levels of chlorogenic acid, however, additional information is required to determine the effect of stress on the synthesis of chlorogenic acid.

Although extremes of 2.2 to 5.1% chlorogenic acid were observed, the range in location, variety and year averages were small and inconsistent, indicating that existing genotype and environmental conditions did not reduce chlorogenic levels enough to be of practical value.

Date of Sowing. Early sowing consistently resulted in the highest accumulation of chlorogenic acid (table 1). Any delay after the third week in May caused a progressive reduction in chlorogenic acid levels. Sowing after June 11 shifted the period of synthesis of phenolics into the cooler portion of the growing season and simultaneously compressing the whole period of growth. All varieties tested responded in a similar manner.

Table 1

Effect of sowing date on the chlorogenic acid content of sunflower meal from the varieties Krasnodarets and Peredovik

Date	% Chlorogenic acid			
	1971	1972	1973	Average
Before May 23	4.1	4.6	3.9	4.2
May 23-May 31	3.8	4.3	3.8	4.0
June 1-June 11	3.7	4.0	3.2	3.7
Later than June 11	3.3	3.6	2.8	3.3
Average	3.8	4.1	3 4	3.8

Synthesis of chlorogenic acid. Seed samples were taken at weekly intervals from 21 to 49 days after blooming. During this period seed moisture dropped from an average of 66% to 21% while chlorogenic

Table 2

Amount of chlorogenic acid present in sunflower meal from seed of the varieties Krasnodarets and Peredovik harvested at various stages after flowering in 1972

Days after flowering	% Chlorogenic acid		Average
	Krasnodarets	Peredovik	
21	1.40 c*	1.47 c	1.43
28	2.93 b	2.30 b	2.62
35	3.60 a	3.06 a	3.33
42	3.76 a	3.14 a	3.45
49	3.84 a	3.32 a	3.58
Average	3.10	2.65	

* Values within columns followed by the same letter are not significantly different at $P = 0.05$.

acid in the meal increased from 1.4% to 3.6% (table 2). There was a rapid increase from 21 to 35 days after flowering followed by a leveling off. This trend is quite similar to the deposition of oil. Studies are underway to determine when synthesis begins, the rate of synthesis in the initial stages and the effect of specific stress factors during synthesis.

Genetic variability. Selection within current North American varieties appears impractical because of limited variability. However, an analysis of 166 lines from the World Collection, grown under the same environment, revealed a range in chlorogenic acid from 1.4 to 4.0% (figure 1). Lines with extreme levels of chlorogenic acid were re-grown and the values found to be consistent with the original analysis.

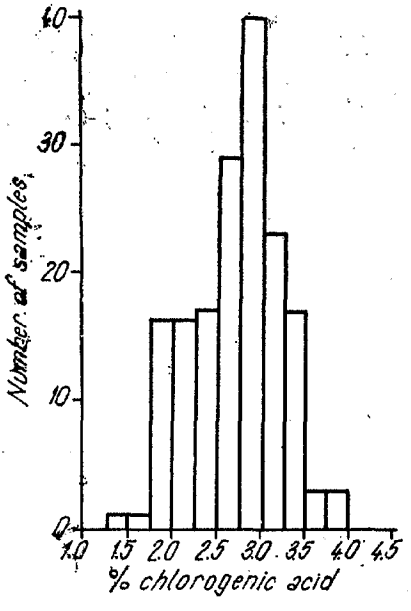

Fig. 1 — Chlorogenic acid content of dehulled, and defatted sunflower meal from inbred lines from the world collection.

Segregating populations from a cross between the variety Krasnodarets (3.8%) and a low phenolic selection (1.5%) contained as little as 1.4% chlorogenic acid. Thus it appears that it will be relatively easy to reduce the level of chlorogenic acid. Additional selection within *H. annuus* and cross-compatible species will be undertaken. Levels of less than 1% may be feasible. It is not known what effect this will have on disease resistance or other reactions reportedly involving chlorogenic acid. If the influence is minimal, this level of phenolics should make any of the patented or proposed methods of extracting phenolics sufficiently economical to encourage the production of high quality sunflower protein.

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