# FACTORS AFFECTING CHLOROGENIC, QUINIC, AND CAFFEIC ACID LEVELS IN SUNFLOWER KERNELS

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## Summary

The location of the seed on the sunflower head and storage temperatures were related to chlorogenic, caffeic, and quinic acid levels. Kernels of freshly harvested sunflower seeds located near the center of the head were higher in chlorogenic acid than seeds located near the margin. The opposite was obtained for the caffeic acid content, and quinic acid did not exhibit a positional effect. Chlorogenic acid content decreased during storage at 5°C, 15°C, and 40°C for 120 days; seeds attained the same levels of chlorogenic acid but varied slightly among treatments. Relatively small changes occured in the caffeic and quinic acids content during storage.

## Introduction

Sunflower seeds are an important commercial source of edible oils and animal feeds. The meal resulting from the oil extraction process is a valuable source of proteins, but its use in the human diet is limited by the presence of chlorogenic acid. The phenol darkens upon oxidation and can impair the acceptability of sunflower products.

Although chlorogenic acid is an important factor in the discoloration of the meal, other factors such as particles of hulls, pH, oxidizing agents, and cooking conditions are also concerned with the discolorations.

Chlorogenic acid functions as part of the oxidase system in the plants (Hanson et al., 1963). Removal of chlorogenic acid with organic solvents has been attempted but without practical success (Smith, 1958). The most efficient solvent was 70% ethanol.

Chlorogenic and quinic acid have been isolated from the kernel of sunflower seeds by column chromotography and identified by paper chromatography. The level of both acids in the kernel and hull of sunflower seed, and the conversion of chlorogenic acid into quinic acid at temperature of

100°C, and 135°C and resynthesis of chlorogenic acid during cold storage of the meal has been reported. (Milic et al., 1968)

Acid hydrolysis of chlorogenic acid results in caffeic acid. This acid is also present in the sunflower kernel. In the study reported here, the concentration of chlorogenic, quinic, and caffeic acid was investigated at different locations on the head and at storage temperatures of 5°C, 15°C, and 40°C.

## Materials and Methods

Freshly harvested sunflower heads of the Krasnodarets variety grown in College Station, Texas were used. Ten heads were selected for homogeneous appearance and freedom from insect damage. The seeds sampled were located within two inches of the center of the head. They were collected and kept separated from subsequent seeds collected from the same heads but located 2 inches from the center to the margin. The two lots of seeds were equally divided for storage treatments at 5°C, 15°C, and 40°C for periods of 0, 60, 90, and 120 days.

At the end of each storage treatment, the seeds were dehulled by violent agitation by means of a jet of comprssed air in a special container. A 12 ounce Ball refrigerator and freezer jar was covered at the top with two layers of 16 mesh wire screen and a pipe 0.5 cm diameter was inserted 1 cm in a small hole at the edge of the wire screen.

Fifty $lb/in^2$ of air was applied for 30 seconds to one minute depending on the size and variety of the sample being dehulled. Fifteen to 20 grams of each sample were dehulled followed by separation of kernels and hulls in an air stream. This method decorticated the seeds quickly with a minimum damage to the kernels.

Kernels were ground in a Waring blender for approximately 20 seconds to pass a 40 mesh screen. They were oven dried at 75°C for six hours and extracted with petroleum ether for 18 hours in a Soxhlet apparatus. Two grams of the fat-free residue were extracted for 14 hours with 80 per cent ethanol in Soxhlet and the extract volume was reduced to 50 ml in vacuo at 40°C.

### Chlorogenic Acid

100 lambda of the ethanol extract was spotted on Whatman No. 1 chromatography paper 2.5 cm above the bottom and over a length of 4 cm and a maximum width of 1 cm. The chromatogram was developed for five hours or until the solvent front moved 14.5 cm. Best results were obtained in small chambers without equilibration periods. The solvent was butanol: acetic acid: water (4:1:5).

Two flourescent spots appeared. One, with $R_f$ value of .60 and bluish color under ultra-violet light, was identified as chlorogenic acid. The spot was removed and quantitatively analyzed by a modification of the Hoepfner reagent (Hoepfner, 1932). The paper was treated with 1 ml of water and 4 ml of 1:1 mixture of freshly prepared 5 per cent acetic acid and 0.5 per cent sodium nitrite; then it was shaken and thoroughly centrifuged

at 2000 rpm for 4 minutes. Absorption was measured with a Beckman DB spectrophotometer at 520 mu. The absorbancy was then compared with a standard curve made of a concentration series and found to be linear over the range of 0.10 to 1.00 umol.

Quinic Acid

2-Keto-3-deoyx-D Arabo-Heptonic acid 7-p Synthetase (KDHP) has a similar molecular structure to that of quinic acid. The method for determination of KDHP (Sirinivasan et al., 1959) was adapted to quinic acid. One mililiter of the ethanol extract was treated with 1 ml of 0.025 N $HIO_4$ in 0.125 N $H_2SO_4$, shaken thoroughly, and let stand for 15 minutes. The excess periodate was removed with 2 ml of 2 per cent solution of sodium arsenite in 0.5 N HCL and shaken until iodine vapors had evolved. After 2 minutes, 2 ml of 0.6 per cent solution of 2-thio-barbituric acid was added. The sample was shaken and placed in a warm bath for 40 minutes. The pink colored solution was diluted to 10 ml with acetone and centrifuged for 3 minutes at 2000 rpm. The absorbancy was measured at 550 mu and compared with a curve of a concentration series found to be linear over the range of 0.10 to 0.64 umol of quinic acid.

## Results and Discussion

Initial analysis of freshly harvested sunflower seed of the Krasnodarets variety revealed that the seed located within 2 inches of the center of the sunflower head contained about twice as much chlorogenic acid as the seeds located 2 inches from the center to the margin of the sunflower head. A possible explanation may be physiological state of maturity. Sunflower seed located toward the margin of the head reach physiological maturity first (Kinman, 1969, personal communication). As a consequence, biological activity such as respiration and enzyme activity would be less. Dramatic changes in phenolic composition occurs during plant development and some phenolic compounds are synthetized for only a brief period during a particular stage of growth (Zucker et al., 1967). For example, it has been shown the concentration of chlorogenic acid at the tip of the tobacco leaf was greater than at the base, and this corresponded to the concentration of substances synthetized in situ such as proteins and chlorophyll (Zucker and Ahrens, 1958). This same postulate could apply to sunflower seeds. The initial content of quinic acid was the same for seeds located near the margin and those located toward the center of the head. Initial content of caffeic acid was higher for seeds located toward the margin than for seeds located toward the center of the sunflower head.

Storage of the seed at 5°C, 15°C, and 40°C for 60 days caused a decrease in chlorogenic acid; this decrease was more pronounced in the seeds located toward the center of the head. After 120 days of storage, the content of chlorogenic acid was similar for seeds that received the same heat treatment. Differences, however, were found between storage heat treatments. The highest level was in the seeds treated at 40°C; next was 15°C; and the lowest was for seeds kept below 5°C.

Values for quinic acid increased after storage at 50°C, 15°C, and 40°C. Leveling off occurred among the seeds that received the same heat treatment

after storage for 120 days. The highest concentration was obtained for seeds treated at 5°C; next was 15°C; and the lowest was found at 40°C.

The values for caffeic acid decreased after storage at 5°C, 15°C, and 40°C for 60 days but increased to the same level among seeds that received the same heat treatment after storage for 120 days. The highest concentration was obtained for seeds treated at 40°C, then 5°C, and the lowest at 15°C. Seeds that received the same heat treatment had similar concentrations of caffeic acid.

Synthesis of chlorogenic acid during cold storage of potatoes has been reported. Glucose, fructose and saccharose accumulate during cold storage of potatoes and are very effective substrates for chlorogenic acid synthesis (Zucker and Levy, 1959). L-Phenylalanine with quinic acid was also found to be an effective substrate for chlorogenic acid synthesis (Hanson and Zucker, 1963).

It was reported (Milic et al, 1968) that a decrease in chlorogenic acid caused an increase in quinic acid in sunflower kernels which had been heat-treated for five hours at 70°C, 100°C, and 135°C. This was followed by an increase in chlorogenic acid upon storage at 20°C. It was not reported, however, if the seeds were able to germinate. This treatment might have killed the seed but, not damaged the mechanism of chlorogenic acid synthesis from the lower degradation complexes of polysaccharides and proteins resulting from the heat treatment. This would explain the increase in the chlorgenic acid level after storage at 70°C for 56 days.

A possible explanation for the changes occurring in the levels of chlorogenic, caffeic, and quinic acids before and after heat treatments would be the selective induction and repression of enzymes by temperature, time, light, substrate availability, or a combination of these factors in phenolic biosynthesis (Zucker et al., 1967).

It can be postulated that immediately after harvesting a breakdown in chlorogenic acid occurred until a steady state was reached. The effect of inducers and repressors such as heat, carbohydrates or enzymes regulated the activity of the phenolic biosynthesis.

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