other. At 165 °C the inhibitors have practically disappeared. The flour turned brown in the same temperature range where the inhibitors were inactivated. This parallelism suggests that the dry heat inactivation is at least partially due to the Maillard reaction.

Experiment 2. The changes in trypsin- and chymotrypsin-inhibitor activities after heating the saline extract of soybean flour are shown in Figure 2. The unheated control extract inhibited approximately twice as much trypsin as chymotrypsin, similar to that in the previous experiment. After the extract was kept at 65 °C for 10 min, protein precipitation was noticed, which was removed by centrifugation. No significant amounts of active inhibitor were regained from the precipitate. At this temperature the chymotrypsin-inhibiting capacity fell steeply, while the trypsin inhibitor did the same though less markedly. Between 65 and 100 °C the chymotrypsin inhibitor did not decrease further, while the trypsin inhibitor declined to the greatest extent in this temperature range.

Comparing the effects of dry and wet heat treatment, it is noteworthy that the inhibitors have a much higher resistance in dry environment than after extraction. In each treatment the most heat-stable fraction inhibited both trypsin and chymotrypsin. This fraction is probably identical with the Bowman–Birk inhibitor, which has a higher heat stability than the Kunitz inhibitor even in purified state. Inactivation of the latter seems to occur in those temperature ranges where only a decrease of trypsin inhibitor is observable.

Experiment 3. The progress of the reaction of trypsin or chymotrypsin with the saline extract of soybean flour is shown in Figure 3. The decline of activity of both proteinases showed similar curves. The reaction was completed in ~10 minutes at room temperature. However, when the solutions stood overnight, the activity of proteinases increases again. This indicates that both trypsin and chymotrypsin can digest a part or some fractions of the inhibitors during longer periods of incubation.

LITERATURE CITED

Birk, Y.; Gertler, A. L.; Khalef, S. *Biochem. J.* **1963**, *87*, 281.
Bowman, D. E. *Proc. Soc. Exp. Biol. Med.* **1946**, *63*, 547.
Kunitz, M. *Science* **1945**, *101*, 668.
Schwert, G. W.; Takenaka, Y. *Biochim. Biophys. Acta* **1955**, *16*, 570.

K. Baintner

Department of Physiology
Research Institute for Animal Breeding
1024 Budapest, Kitaibel-u. 4, Hungary

Received for review August 29, 1977. Resubmitted April 4, 1980. Accepted September 2, 1980.

Changes in Chlorogenic, Caffeic, and Quinic Acid Contents during Sunflower Seed Maturation

Changes in chlorogenic (CGA), caffeic (CA), and quinic (QA) acid contents in sunflower seeds (EC 68415) were determined during seed maturation. CGA content increased slowly from 21 to 28 days after flowering, rapidly from 28 to 35 days, and again slowly up to 43 days when the CGA content was maximum. CA decreased appreciably and QA slightly from 21 to 28 days, and thereafter CA decreased slowly and QA rapidly up to 43 days. From then until 49 days, CGA decreased to a small extent while CA and QA increased appreciably. Variations in CGA, and CA, and QA during seed maturation thus appear to be interrelated.

The presence of chlorogenic acid (CGA) as well as its components, caffeic (CA) and quinic (QA) acids, in sunflower seeds is well-known (Sechet et al., 1959; Milic et al., 1968). The presence of CGA is not desirable since it causes discoloration of proteins at alkaline pH (Smith and Johnsen, 1948). CGA is reported to vary with the position of seed in the flower head due to differences in the physiological state of maturity (Pomenta and Burns, 1971) and with cultivar (Brummet and Burns, 1972). However, information on variations in the contents of CGA as well as CA and QA with seed maturation is lacking, and results of such an investigation are reported here.

MATERIALS AND METHODS

Samples of sunflower seeds *Helianthus annuus* L. (var. EC 68415) were collected from plants grown on a bulk plot in the fields of Hayathnagar Research Farm (ICAR), Hyderabad, at different stages of seed maturation from 21 to 49 days after flowering, which occurred 60 days after planting. A composite seed sample (~40 g) was prepared by mixing all the seeds from the flower heads of 20 plants selected at random. The seeds were decorticated, and oil was extracted from the kernels with *n*-hexane in a Soxhlet apparatus. The defatted meal was desolventized by spreading in a thin layer and exposing to the atmosphere for 8 h at room temperature (30 °C). Standard procedures used for the analysis of sunflower seed deoiled meals, namely, those of Sosulski et al. (1972) for CGA and CA contents and of Mesnard and Devaux (1964) for QA content, were followed. Absorption was measured at 324 and 320 nm for CGA and CA, respectively (Cater et al., 1972). Standard curves were prepared for CGA and CA (Cater et al., 1972) and for QA (Mesnard and Devaux, 1964) from chemicals (over 96% pure) purchased from Sigma Chemical Co., St. Louis, MO, USA after drying them overnight at 50 °C under vacuum. The purity of these chemicals was determined by comparison with pure samples isolated by preparative thin-layer chromatography on 1 mm thick silica gel G using butanol–acetic acid–water (4:1:5 v/v).

RESULTS AND DISCUSSION

The relative changes in contents of CGA, CA, and QA in the defatted meal samples from seeds at different stages of maturation are given in Table I. The data show that the CGA content increased with progressive maturation of the seed, gradually from 21 to 28 days after flowering,

0021-8561/81/1429-0203$01.00/0

Table I. Changes in Chlorogenic, Caffeic, and Quinic Acid Contents during Sunflower Seed Maturation

days after flowering	g/100 g[a] chlorogenic acid	caffeic acid	quinic acid
21	0.13	0.17	0.52
28	0.24	0.13	0.48
35	1.05	0.12	0.33
43	1.09	0.11	0.26
49	0.87	0.15	0.39

[a] In decorticated and defatted sunflower (EC 68415) seeds, g/100 g on a moisture-free basis (average of duplicates which varied within 5%).

rapidly from 28 to 35 days, and only slightly from 35 to 43 days. On the same 43rd day, the oil content in the seeds and seed index (g/100 dry seeds) also reached maximum values, 45.4% and 6.13, respectively (Afzalpurkar and Lakshminarayana, 1979). There was some reduction in CGA from 43 to 49 days. CA decreased initially (from 21 to 28 days) to some extent and then slightly thereafter up to 43 days but increased from 43 to 49 days. QA decreased to a small extent in the initial stage (from 21 to 28 days) and then rapidly thereafter until 43 days but increased substantially from 43 to 49 days.

The relative changes observed in CGA, CA, and QA contents may not be biological variations since each sample was obtained from a pool of seeds from 20 plants at the same stage of seed maturation. The increase in CGA content up to 43 days and decrease in the contents of CA and QA during the same period indicate the possibility of formation of CGA from CA and QA during seed maturation. Decrease in CGA content and increase in CA and QA contents from 43 to 49 days indicate either hydrolysis of CGA into CA and QA or a slower rate of synthesis of CGA compared to the syntheses of CA and QA due to differences in the reduction of the enzyme activities during the final stage of seed maturation. Interconversions leading to the changes in the levels of these constituents during storage at different temperatures were reported by earlier workers and attributed to the variations in the enzymatic activity (Milic et al., 1968; Pomenta and Burns, 1971). Interconversions between CGA and CA and QA also appear to occur during seed maturation.

ACKNOWLEDGMENT

Thanks are due to Dr. J. Venkateswarlu, All India Co-Ordinated Research Project for Dryland Agriculture (ICAR), Hyderabad, for seed samples.

LITERATURE CITED

Afzalpurkar, A. B.; Lakshminarayana, G. *J. Oil Technol. Assoc. India* **1979**, *11*, 83.
Brummet, B. J.; Burns, E. E. *J. Food Sci.* **1972**, *37*, 1.
Cater, C. M.; Gheyasuddin, S.; Mattil, K. F. *Cereal Chem.* **1972**, *49*, 508.
Mesnard, P.; Devaux, G. *Bull. Soc. Chim. Fr.* **1964**, *1*, 43.
Milic, B.; Stojanovic, S.; Vucurevic, N.; Turcic, M. *J. Sci. Food Agric.* **1968**, *19*, 108.
Pomenta, J. L.; Burns, E. E. *J. Food Sci.* **1971**, *36*, 490.
Sechet, J.; Masquelier, J.; Tayeau, F. *Bull. Soc. Chim. Biol.* **1959**, *41*, 1067.
Smith, A. K.; Johnsen, V. L. *Cereal Chem.* **1948**, *25*, 399.
Sosulski, F. W.; McCleary, C. W.; Soliman, F. S. *J. Food Sci.* **1972**, *37*, 253.

Ashok B. Afzalpurkar
Gollamudi Lakshminarayana*

Regional Research Laboratory (CSIR)
Hyderabad 500 009, India

Received for review February 20, 1980. Accepted October 14, 1980.